INHERITANCE OF GROWTH HABIT AND PHOSPHOGLUCOISOMERASE ISOZYMES IN *PENNISETUM ALOPECUROIDES* (L.) SPRENG.

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MARY HOCKENBERRY MEYER

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This is to certify that I have examined this bound copy of a doctoral thesis by

Mary Hockenberry Meyer

and have found that it is complete and satisfactory in all respects, and that any and all revisions required by the final examining committee have been made.

Donald B. White

Name of Faculty Advisor

Donald B. White

Signature of Faculty Advisor

11 June 1993

Date

GRADUATE SCHOOL

This thesis is dedicated to my parents,

William Wadsworth Hockenberry

and

Thorene Anderson Hockenberry

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ABSTRACT

Fountain grass, Pennisetum alopecuroides (L.) Spreng., is a C_4 bunch type perennial typically 90-125 cm tall with numerous purple or mauve spike-like inflorescences. It has been grown as an ornamental in the U.S. since the early 1940's, yet only one form is readily available in the trade. Evaluation of germplasm from several sources resulted in the identification of four growth habits classified as prostrate (p), upright (u), mound (m), and dwarf (d). No reports of breeding improvements or basic genetics could be found for fountain grass. On that basis, three areas of research were selected: inheritance of growth habit, isozyme variation and inheritance, and pollen viability.

Selected plants of the four growth habits were crossed in a complete diallel in 1990 and 1991. F_1 and F_2 progeny evaluations support the conclusion that the dwarf and upright forms appear to share similar genetic backgrounds, as do the mound and prostrate forms. The dwarf and prostrate forms appear to be controlled by one or two recessive genes. The upright and mound appear to be dominant traits. Progeny from four crosses, d x p; p x d; m x d; and d x m, exhibited heterosis in culm length, exceeding either parent.

Ten isozymes were screened for variation. Polymorphism was found only in phosphoglucoisomerase at one locus, PGI-2, and appeared to be associated with growth habit. The dwarf form exhibited one slow band, SS; extracts from the mound and prostrate forms yielded one fast band, FF, and the upright formed triple bands, FS, a heterodimer. Hybrids between FF and SS parents could be detected as triple bands, FS. Three generations followed expected segregation ratios for this isozyme.

In vitro pollen germination (in a 25 g kg sucrose and 100 mg ml boric acid solution) ranged from a low of 4% for the F_1 mound progeny to a high of 47% for the upright parent.

Fountain grass appears to have sufficient genetic diversity for future selection and improvement as an ornamental.

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INTRODUCTION

Fountain grass, P.alopecuroides (L.) Spreng., is an attractive ornamental that has not been researched or improved for horticultural traits. Preliminary investigations revealed four growth habits, which were identified as prostrate, upright, mound and dwarf.

This research was undertaken to evaluate the variation and inheritance of these growth habits, as well as isozyme variation, and pollen viability in fountain grass.

Hypotheses

The absence of literature on growth habit, breeding behavior and isozyme variation of fountain grass dictated a need for investigation into these areas. On that basis this research was guided by the following three hypothesis and objectives:

Hypothesis I:

Isozyme analysis will show sufficient specificity for identification of intraspecific hybrids.

Objective I:

a. To establish a set of protocols for starch gel electrophoresis with fountain grass.

b. To determine if intraspecific hybrids can be identified through isozyme analysis.

c. To determine if phenotypic variation is reflected in isozyme variation.

d. To determine if selected enzyme systems express sufficient specificity for genetic analysis.

Hypothesis II:

Four selected growth habits: 1)prostrate, 2)upright, 3)mound, and 4)dwarf in fountain grass are largely controlled by 1 or 2 genes.

Objective II:

To investigate the inheritance of the genetics associated with four growth habits. This will be accomplished by crossing these forms in

a complete diallel matrix and analyzing the data related to inheritance.

Hypothesis III

Pollen viability asociated with plants of the four growth habits can be determined by in vitro germination.

Objective III:

a. Establish a technique for in vitro germination of fountain grass pollen.

b. Determine viability by % pollen germination in plants of the four growth habits and their F_1 progeny.

Significance

Fountain grass is an attractive ornamental that has not been improved or studied for diversity, although several floras in the Far East cite its extensive range and wide adaptation to a variety of habitats.

This research will provide basic genetic information on the inheritance of growth habit, which may enable the development of new ornamental cultivars and has wide application to.other grasses, whether turfgrass, ornamentals, forage or cereals.

Isozymes are usually under simple genetic control, typically monogenic with codominant expression of alleles at a single locus and as such, inheritance studies are often straight forward (Tanksley and Orton, 1983). Conversely, traditional breeding methods that involve the selection and crossing of individuals based on gross phenotypic differences may present a much more complex inheritance study involving several genes with different levels of expression.

Using a traditional breeding method in combination with a newer biochemical technique may reveal relationships or patterns such as isozyme markers that correspond to specific phenotypes.

Pollen viability is critical in any breeding program. If fountain grass sets seed predominantly by outcrossing, as appears likely given the protogynous flowers, inbreeding may negatively affect pollen

viability. A reliable method for screening viability is necessary to determine this effect.

It is hoped that these hypotheses will form a sound basis for a breeding program that will provide further understanding of the genetics of growth habit and isozyme variation in fountain grass.

LITERATURE REVIEW

Pennisetum is a large genus of about 130 warm season grasses in the Paniceae tribe of the Poaceae family (Hitchcock,1949).

P.alopecuroides (L.) Spreng., fountain grass, is typically a bunch type perennial, with numerous culms, 90-120 cm tall, with purple or mauve, spike-like inflorescences. As an ornamental, fountain grass has been grown in the U.S. since the early 1940's, (Bailey 1949) however its use has been limited to one form (Meyer,1975; Smithburg,1960). It is reported to be a diploid, 2n = 18, (Ono,1953).

Its natural range in the Far East extends from 48° N latitude in Manchuria (Kitagawa, 1979) to 38° S latitude, along the coast of New South Wales in Australia (Wheeler,1982). Floras from Japan,(Ohwi,1965; Okuyama,1982); Burma (Bor, 1960); China (Steward,1958; ICS,1976); the Okinawa Islands (Walker,1976); and Australia (Black,1978; Cunningham, 1981) all list fountain grass as native. Habitat varies from coastal areas (Cunningham, 1981), boggy, wet lowland soils (Bor,1960; Ohwi, 1965;Black,1978); to open plains (Bor,1965) and inland mountains (Kitagawa, 1979). Walker (1976), cites the following forms:

forma alopecuroides inflorescence brownish, spikelets dense.

forma viridescens (Miq.) Ohwi; inflorescence pale-green; spikelets loose.

And from Ohwi (1965):

forma purpurascens (Thunb.)Ohwi; bristles dark purple; the commonest form.

forma viridescens (Miq.) Ohwi; bristles pale green.

forma erthrochaetum Ohwi; bristles red.

The common name for fountain grass in China is Wolf's Tail Grass; in Japan, Chikara-Shiba, which translates to strong or vigorous lawn grass; and in Australia, Swamp Foxtail.

Germany is thought to be the source of two dwarf commercial cultivars 'Hameln' and 'Weserbergland'(Foerster,1982) which are rarely found in the U.S. Foerster (1982) reported that the larger Australian form was superior, especially in hardiness, to the Japanese form. In the U.S., usually the large upright is the only form readily available.

With its wide range of adaptation and variation listed in floras, other forms of fountain grass should be examined for variation and value as ornamentals.

Due to a lack of information on fountain Grass, pearl millet, Pennisetum glaucum (L.) R.Br., other species of Pennisetum, as well as other genera will be reviewed on the subjects of interest for this research.

Isozymes

Isozymes may be useful in documentation of variation between growth habits of fountain grass. If the different growth habits have distant geographic origins this may be expressed as variation in isozymes (Brown and Weir,1983). To our knowledge, there are no reports of isozyme research with this species. Therefore, polymorphism in pearl millet and phosphoglucoisomerase (PGI), which was found to be variable in fountain grass will be reviewed.

Isozymes, first defined by Markert and Moller (1959), are two or more distinguishable enzymes that catalyze the same biochemical reaction. Isozyme analysis has been used to identify and discriminate among inbreds and hybrids, (Goodman and Stuber,1983); to study phylogenetic and evolutionary patterns within a species (Tostain,et.al.,1987); to estimate gene frequency (Hamrick,1989); to

associate specific alleles with agronomically useful traits (Stuber,1989); to identify species and cultivars (Cousineau and Donnelly,1992); to separate and classify genotypes (Tanksley and Orton,1983) and to access genetic diversity and polymorphism (Weeden,1988).

Several major reviews and books have been written on isozymes including Tanksley and Orton (1983), Scandalios and Whitt (1985); Weeden (1988), Soltis and Soltis (1989).

Baruett-Bourrillon and Hague (1979) reported on variation in alcohol dehydrogenase (ADH), in pearl millet. They found two linked genes which specified 3 sets of ADH isozymes. Baruett-Bourrillion (1982) also found naturally occurring variants which had altered gene activity for one set of ADH isozymes.

Sandemeir et. al.(1981) reported that esterase was a dimeric protein with nine different alleles in pearl millet.

Leblanc and Pernes (1983) recorded the enzyme structure and number of loci encoding for ADH (dimer, 1 locus), phosphoglucomutase (PGM, monomer, 1 locus), and PGI (dimer, 1 locus) in pearl millet. They discussed variation in populations from the Northern Ivory Coast of Africa and found a "significant linear variation of frequencies associated with an east west axis" for the two loci encoding for PGM and ADH. They proposed two hypotheses to account for this evolutionary divergence in isozymes. The first hypothesis was that the divergence was the effect of isolation and limited genetic flow; the second was that environmental conditions (flooding) favored plants with specific enzyme systems from east to west.

Tostain and Riandey (1984), analyzed polymorphism and the genetics and number of genes coding for alcohol dehydrogenase (ADH),catalase (CAT), and esterase (EST) in pearl millet. They also reported (1985) that malate dehydrogenase (MDH) was composed of several dimeric enzymes under complex genetic control, and located in the mitochondria and

cytosol.

Tostain (1985) evaluated linkage relations between the dwarfing D_2d_2 and 7 enzymatic marker genes in pearl millet. He found a linkage between two genes for PGI and PGM (labeled Pgi A and Pgm A), between two genes of shikimate dehydrogenase and ADH (Skdh A and Adh A), between D_2 and Skdh A, and between D_2 and Adh A. The latter three genes are linked in the following order:

Adh A - 11 centimorgans - Skdh A - 9 centimorgens - D_2.

Thus most dwarf plants tested had isozyme markers for Adh A and SkdhA. It was suggested that the heterozygote (D_2d_2) could be screened at the seedling stage.

Trigui, et. al. (1986) observed 12 enzyme systems in pearl millet and proposed inheritance of these systems based on the zymograms of the parents and their F_1 hybrids. They reported PGI to be a dimer with five different alleles found at one locus.

Tostain, et. al. (1987), Tostain and Maichais (1989), and Tostain (1992) studied enzyme diversity in nearly 200 strains of wild and cultivated pearl millet. They divided the strains into four groups (early maturing, late maturing, cultivated-India, and cultivated-South Africa) based on eight enzyme systems: ADH, CAT, EST, glutamate oxaloacetate transaminase (GOT), MDH, phosphogluconate dehydrogenase (PGD), PGM, PGI. They submitted an evolutionary hypothesis for the domestication and migration of early versus late maturing forms based on enzyme diversity.

In addition to the report by Tostain (1985) above, a few other observations of linkage between isozyme markers and phenotypic agronomic traits have been reported. Linkages still remain the exception rather than the rule. Disease resistant genes have been linked with isozyme markers in wheat (McMillin et.al.,1986), garden pea, squash (Weeden and Marx, 1984), and tomato (Rick and Forbes,1974). Isozymes have also been identified as markers for male sterility (Tanksley et.al.,1984), self-

incompatibility (Wricke and Wehling,1985), cold tolerance (Vallejos and Tanksley,1983) and yield (Pollack et.al.,1983). Kusmenoglu et.al. (1992) reported a loose linkage between the gene controlling growth habit in chickpea and the gene encoding for the cytosolic form of 6-phosphogluconate dehydrogenase (PGD-c).

Gottlieb (1982) reviewed the subunit structure, number of loci and subcellular localization of isozymes, especially those catalyzing steps in primary metabolism such as PGI, and observed that these characteristics are well conserved in plant species.

The dimeric enzyme structure of PGI has been demonstrated in several plants including guayule, (Hashemi,et.al.1991); tomato, (Tanksley,1980); tepary bean, (Garvin,et.al.1989) spinach (Schnarrenberger and Oeser,1974), sweet potato (Reyes and Collins, 1992) and corn (Goodman and Stuber, 1983) and pearl millet (Lablanc and Pernes, 1983; Trigui, et.al.,1986).

Most references have found two loci encoding for PGI. The faster migrating enzymes, usually labeled PGI-1, are nearer the anode, and are found in the plastids. PGI-2 forms slower migrating extracts and is found in the cytosol. PGI-1 tends to be monomorphic and PGI-2 is often polymorphic.

Growth Habit Genetics

Burton (1983) indicated that many important characters in pearl millet are largely controlled by a single dominant or recessive gene. Burton and Fortson (1966), studied five different dwarf inbred lines of pearl millet and reported that dwarfness in 2 lines was largely conditioned by two different recessive genes which they named d_1 and d_2 respectively. However, the 3 other inbreds had two or more genes which accounted for their dwarfness. They also found most dwarf x normal F_1 hybrids were significantly taller than their normal parents. The d_2 gene has been reported to shorten all internodes except the peduncle, reduce plant height 50%, and increase leafiness and forage quality in pearl

millet (Burton,1983).

Krisha Rao, et. al. (1981) found the d_2 gene in Tift 23DB to be defective in the use of gibberellic acid, GA_3. Not only was GA_3 detected in the dwarf plants but there was no response to exogenous application of GA_3.

Soman, et.al (1989) reported the d_2 gene in pearl millet did not affect the coleoptile or mesocotyl length, however the culm length differed significantly between tall and dwarf genotypes.

Rai and Hanna (1990) measured the effect of the d_2 dwarfing gene on several morphological characters by comparing tall and dwarf near-isogenic lines. Dwarf isogenic lines were shorter than their tall counterparts, but had larger peduncles, longer and narrower panicles, thicker culms, wider leaves and smaller seeds.

Appa Rao, et. al. (1986) described 13 new sources of dwarfing genes in pearl millet. In 10 crosses with tall lines of millet, the F_2 segregation was 3 tall:1 dwarf, indicating dwarfness was controlled by a single recessive gene. The remaining 3 F_2 lines showed continuous variation for plant height, indicating more than 1 gene is involved. Two of the newly identified dwarf lines were found to be nonallelic to the d_1 and d_2 genes previously reported and these were assigned the symbols d_3 and d_4.

Kumar and Andrews (1993) reviewed 167 studies on the genetics of qualitative traits in pearl millet. They cite nine references to plant form, the dwarfs discussed above, as well as reports of thick and wavy stem traits.

In sorghum, four independent recessive genes have been identified and designated as dw_1, dw_2, dw_3, dw_4. The dw_3 gene is unstable and can revert to the dominant form causing a tall plant (Poehlman,1987). Therefore, most dwarf sorghum hybrids must carry more than one recessive gene to produce stability.

In other crops, Ladizinsky (1979) studied tall, bushy and

prostrate growth habits in lentils. He concluded that the prostrate growth habit was governed by a single gene with incomplete dominance, and that further study was needed to determine the genotype of the bushy form.

Ozminkowski et.al. (1990) reported that prostrate growth habit in tomato was controlled by more than one gene and expression was influenced by the environment. Elkind et.al.,(1991) found the semideterminant growth habit in tomato was controlled by two genes, one epistatic and the other a single recessive gene which confers the semideterminate habit.

Kusmenoglu (1990) confirmed previous reports that erect growth habit is conditioned by a single dominant gene to semiprostrate in chickpea.

Other reports of dwarf growth habits involve a physiological study of dwarfness, often in relation to gibberellic acid, rather than the inheritance of this trait.

Reproductive Biology

Other than chromosome number, the only reports of fountain grass concern photoperiodic response and although not directly related to this research, they will be reported here. Garner and Allard (1940) included this species in their classic work on photoperiod. They reported flowering in 55 and 83 days with a 10 and 15 hr daylength respectively. Evans (1964), reported this as a short day response, while Cooper (1960) interpreted it as indifferent. Nada (1980), reported flowering at 15 hour days and no flowers at 9 or 12 hr days. However, even under the 15 hr day, heads did not emerge in less than 160 days, which he interpreted as a "relatively long" response.

Fountain grass is protogynous, the stigmas precede anther exertion and dehiscence by 2-3 days (unpublished data, Meyer). Protogyny is typical of other Pennisetum species, such pearl millet and crimson fountain grass Pennisetum setaceum (Forsk.) Chiov., (Burton, 1980;

Jauhar, 1981). This is a natural means of fostering cross pollination, and estimates are for 69% and 82% natural crossing in pearl millet (Burton, 1974).

Pollen Viability

Pollen viability in Poaceae has been estimated by using vital stains (Simpson and Bashaw, 1969), in vitro germination and in vivo tube growth (Helsop-Harrison et.al.,1984), and seed set (Cooper and Burton 1965). Although it can be argued that the ultimate functional test ought to be fertilization, as measured by seed set (Helsop-Harrison et.al. 1984), incompatibility reactions and other phenomena may interfere with pollen function (Stanley and Linskens,1974). Therefore other simpler means for assessing pollen viability have been sought.

Poor correlation has been found with stainability and in vitro germination. Although the flurochromatic reaction (FCR) shows the best correlation with in vitro germination, FCR tests for integrity of the plasmalemma, not pollen viability (Heslop-Harrison,1984). In vitro germination is considered the standard and although it may show false negatives, it is simpler than seed set correlation (Heslop-Harrison,1984).

In vitro germination of Poaceae pollen has been cited as difficult due to the trinucleate nature of the pollen (Mulcahy, 1984). However, Vasil (1960) reported success with in vitro pollen germination of pearl millet in a sucrose boron solution. Chaudhury and Shivanna (1986) had similar success, although they found little correlation between in vitro germination and actual seed set. Hill (1991) found in vitro germination of pearl millet and Pennisetum orientale L. to be preferred over iodine and FCR screening methods.

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For Hort Science

INHERITANCE OF PHOSPHOGLUCOISOMERASE IN FOUNTAIN GRASS[1]

M. Hockenberry Meyer, D. B. White[2]

Dept. of Horticultural Science
University of Minnesota,St. Paul, MN

ABSTRACT

Starch gel electrophoresis was used to screen ten enzyme systems for variation in plants exhibiting four different growth habits: prostrate (p), upright (u), mound (m) and dwarf (d), of Pennisetum alopecuroides (L.) Spreng.,fountain grass. Only phosphoglucoisomerase (PGI; E.C. 5.3.1.9) was found to be polymorphic at one locus, PGI-2, and was expressed as two alleles. The alleles appear to be associated with growth habit. The dwarf form expressed one slow band (SS), the mound and prostrate forms exhibited one fast band (FF), and the upright carried triple bands indicating a heterodimer (FS). Hybrids between FF and SS parents could be detected as triple bands (FS). Three generations of progeny resulting from 16 crosses of these growth habits all followed the expected segregation ratios for typical Mendelian inheritance of this isozyme. Further analysis is needed to determine if linkage exists between the PGI-2 locus and the gene(s) controlling the dwarf growth habit.

Fountain grass is an attractive ornamental, typically 90-125 cm tall with numerous purple or mauve inflorescences that give the plants the appearance of a fountain in August and September (Bailey,1949; Meyer,1975) Although its popularity has increased in the last 10 years, no records of any breeding research or improvement have been reported except the chromosome number, 2n = 18 (Ono, 1953).

[1] Paper No. _____ of the Scientific Journal Series, University of Minnesota Agricultural Experiment Station.
[2] graduate student and professors, respectively.

Because fountain grass is native over a large area [from northern Manchurai (Kitagawa, 1979) southward to New South Wales in Australia (Wheeler,1982)], it is likely that many forms and variants exist.

In 1987, a breeding program for fountain grass was initiated at the University of Minnesota with the goal of selecting new ornamental forms. Initial accessions revealed four growth habits which were identified as upright, dwarf, mound and prostrate.

Isozymes offer several applications in studying germplasm collections: such as separation and classification of genotypes (Tanksley and Orton, 1983); plant identification (Cousineau and Donnelly,1992); tagging of important economic traits (Rick and Forbes,1974) and studying phylogenetic relationships within a species (Tostain,and Marchis,1989).

The purpose of this research was to use isozyme analysis to further classify the variation in fountain grass germplasm and to determine isozyme inheritance.

METHODS AND MATERIALS

Seeds or plants of fountain grass were acquired from several sources. Material recieved as plants was field grown in a Waukegan silt loam soil in St. Paul, MN or in a Hayden loam soil at the University of Minnesota Landscape Arboretum in Chanhassen, MN. Seeds were germinated in the greenhouse and seedlings transplanted to the field.

All plants were evaluated for variation by measuring 13 morphological characters. After two years, four growth habits: prostrate, upright, mound, and dwarf, were identified. Detailed descriptions of these growth habits and their inheritance have reported (Meyer, et.al. 1993).

The prostrate and mound plants were received as seed from the Germplasm Lab in Experiment, Ga. with the origin labeled Korea. The plants identified as upright were received as plants from K. Bluemel Nursery in Baldwin, MD and labeled Pennisetum alopecuroides. The dwarf

form came from John Greenlee Nursery in Pomona, CA, and was labeled Pennisetum alopecuroides 'Weserbergland'.

Four individual plants, one of each growth habit, were selected for use as parents and crossed in a complete diallel during August 1990 and 1991. Fountain grass is very protogynous, the stigmas precede anther exertion and dehiscence by 2 to 3 days, (Meyer, unpublished data). Therefore emasculation was not practiced. Inflorescences were bagged before stigmas were visible. For crosses, the appropriate pollen was applied when stigmas were observed to be receptive and after pollination the heads were immediately rebagged.

Approximately one month after pollination, the inflorescences were collected, the seed was hand cleaned and weighed.

In early spring, at least 50 randomly selected seeds per F_1 line were germinated on blotter paper and then transplanted to cell packs in the greenhouse. In late May seedlings were field planted in a Waukegan silt loam with 1m x 1m spacing and a randomized design by lines. The plants were maintained with normal cultural practices such as weeding, irrigation and fertilization.

F_2 seeds were obtained by bagging a minimum of 5 random heads per F_1 line prior to stigma emergence. At maturity the inflorescences were collected, seeds were cleaned, weighted and the following spring the F_2 plants were grown as outlined above.

F_3 seed were obtained by bagging a minimum of five random inflorescences on F_2 plants prior to stigma emergence. Seeds were collected and cleaned as described above.

Seed from every inflorescence from each plant was handled and tested separately.

A total of 16 F_1 progeny lines from 1991, 16 F_2 lines and 14 F_3 lines (two lines failed to set seed) were screened for isozyme variation and inheritance.

For electrophoresis, approximately 1600 individual dry seeds were

crushed with a glass rod in ceramic wells containing approximately 0.1 ml of refrigerated extraction buffer as described by Leblanc and Pernes (1983). Young leaf tissue was used to screen F_2 plants from the dwarf parent, otherwise only seeds were used for isozyme analysis in this report. The electrophoretic buffer, stain and gel recipes were as described by Morden et.al (1987) and Cardy et.al. (1983), but modified by using potato in place of corn starch. The extracts were absorbed onto paper wicks (2 mm x 11 mm, Whatman 3MM chromatography paper) that were loaded into the starch gels.

Electrophoresis was conducted at 4C at 17 watts or about 300 volts. After approximately 4 hr. the gels were sliced and stained.

Designation of isozymes was based on the migration distance from the origin of the extracts. The fastest migration (most anodal) enzyme was designated with the code F. Seed from the prostrate plant (the F_1,FF) was used as the control in most gels.

Inheritance analyses were conducted using a X^2 goodness-of-fit test assuming monogenic and codominant expression of alleles at a single locus.

RESULTS

Alcohol dehydrogenase (E.C.1.1.1.1), catalase (E.C.1.11.1.6), esterase (E.C.3.1.1),malate dehydrogenase (E.C.1.1.1.40), phosphogluconate dehydrogenase (E.C.1.1.1.44), and phosphoglucomutase (E.C.5.4.2.2), were monomorphic for all plants tested, (Fig.1). Acid phosphotase (E.C.3.1.3.2), glutamate oxaloacetate transaminase (E.C.2.6.1.1), and isocitrate dehydrogenase (E.C.1.1.1.42) were difficult to resolve and require further investigation.

PGI exhibited two zones of activity, the one closest to the anode labeled PGH-1, was monomorphic and often very light (Fig.2); the second, furthest from the anode, labeled PGI-2, was polymorphic and represented two alleles, (Fig.2). The banding pattern of PGI-2 consisted of either one or three bands, which suggests a dimeric enzyme structure. In the

original parents, seed from the dwarf plants expressed one slow band, SS; mound and prostrate F_1 seed exhibited one fast band, FF; and extracts from seeds of the upright plant formed a triple band, FS, or a heterodimer, indicating the heterozygote, FS.

F_1, F_2 and F_3 progeny from selfs and crosses between the four growth habits, all 46 lines tested (2 lines were unable to be tested due to failure to set seed), showed the expected segregation patterns for simple monogenic inheritance at the PGI-2 locus, (Table 1). Intraspecific hybrids could be identified in some crosses as well as selfs.

Although all dwarf plants screened by using young green tissue expressed the SS alleles, the vast majority of individuals tested were as dry seed, their phenotype was unknown.

All F_1 and F_2 seed from prostrate plants formed one fast band, FF. Although several heads were bagged, F_2 prostrate plants failed to set seed, so there was no F_3 generation. Fountain grass seed set varies greatly, perhaps due to environmental conditions, seasonal variation, or incompatibility.

P x u (FF x FS) and the reciprocal cross resulted in a 1FF:1FS segregation in the F_1. F_2 seeds either segregated 1FF:2FS:1SS from a parent with FS alleles, or all progeny carried only FF alleles from a similar parent. In the u x p F_3, all 25 seed screened expressed only the F alleles. Only two seed resulted in the p x u F_3, not enough to verify segregation.

In the p x m (FF x FF) cross and reciprocal, all progeny had only FF alleles for three generations as expected.

The p x d (FF x SS) cross and reciprocal resulted in all FS individuals in the F_1, and the expected 1:2:1 segregation in the next generation (Fig. 3). Seed from the plant screened for the F_3 also segregated 1FF:2FS:1SS. Although still within an acceptable level, (P = .38 and .16) the ratio of FS to SS in these F_3 generations (of the

crosses p x d and F_2 d x p) generations was high.

In the upright (FS) selfed, seed from six progeny plants over the two generations were screened, only one carried the FF alleles, the other five were heterozygotes, FS, and the seed segregated in a 1:2:1 ratio. High, yet acceptable (P = .16) levels of FS individuals were counted for the F_2 indicating the heterozygote may be favored.

Progeny from the u x m (FS x FF) and the reciprocal cross segregated 1FF:1FS and all progeny screened in the F_2 and F_3 generations carried only the FF alleles.

The u x d (FS x SS) and d x u progeny segregated 1FS:1SS (except for 1 self), and the F_2 and F_3 plants selected were heterozygotes with progeny that segregated 1FF:2FS:1SS.

The mound (FF) growth habit yielded only progeny with FF alleles as expected, in the first two generations. However F_3 seeds segregated 1:2:1, indicating that the parent (an F_2 plant) must have been heterozygous for these alleles and may have resulted from outcrossing.

Seed collected from the m x d (FF x SS) cross segregated 11 selfs (FF) to 19 hybrids (FS), indicating that although protogynous, selfing does occur in fountain grass. All individuals tested in the reciprocal cross, d x m, were hybrids (FS). Segregation in the next two generations was as expected, a 1:2:1 in the F_2 and plants screened in the F_3 carried only FF alleles.

All F_1 progeny from the dwarf expressed only SS alleles. In the F_2, 92 individuals were screened, all were expected to carry only SS alleles, but 11 were detected as FS. Of these 11, six were seeds selected randomly from selfs, and five were plants that were identified as non-dwarf (phenotypically distinct with coarse foliage and taller culms than the other dwarfs) in the F_2 population. In the zymogram for 25 of these putative selfed dwarfs, the four non-dwarfs can be identified as heterodimers, Fig.2. The remaining F_2's were 30 dwarf plants that carried the two SS alleles. Although many heads were bagged,

only 6 seed were obtained for the F_3 generation, too few to verify segregation.

DISCUSSION

PGI appears to be a dimeric enzyme in fountain grass. PGI-2, as analyzed here corresponds to the cytosolic locus of this enzyme which has been confirmed in several plants including corn (Goodman and Stuber, 1983); guayule, (Hashemi, et.al.,1991); pearl millet, (Leblanc and Pernes,1983); tomato, (Tanksley and Rick, 1980); tepary bean, (Garvin et.al.,1989); and spinach (Schnarrenberger and Oeser,1974). These examples reinforce Gottlieb's (1982) review that subunit structure, number of loci and subcellular localization of isozymes, especially those catalyzing steps in primary metabolism such as PGI in glycolysis, are well conserved in plant species.

The SS band at the PGI-2 locus of fountain grass appeared in all phenotypically dwarf plants that were tested. Cases of linkage between enzyme genes or isozyme markers and phenotypic traits are known, but are still the exception rather than the rule (Weeden,1988). Tostain (1985) investigated linkage between the dwarfing gene, D_2d_2, and seven enzymic marker genes including PGI. He found a linkage between shikimate dehydrogenase (Skdh A) and the recessive dwarfing gene d_2d_2. Kusmenoglu, et.al.(1992) reported a loose linkage between the gene controlling prostrate growth habit and the gene encoding for the cytosolic form of 6-phospho-gluconate dehydrogenase (Pgd-c) in chickpea.

In five lines of F_2 progeny (u selfed; m x d; d x u; d x p; d x m) and one F_3 progeny (p x d) there were high levels of heterozygotes (FS) although the ratios fit X^2 expectations. Weeden and Wendel (1989) discuss skewed segregation ratios where isozyme loci were "closely linked to genes or chromosomal segments exposed to strong selection pressures during gametophytic and postzygotic development." Mitton (1989) reported that "at least some enzyme polymorphisms have a substantial impact on plant physiology" and other reports suggest that

heterozygous individuals can capture the highest fitness (Rainey,et.al.1987) and may be favored to compete and survive over homozygotes.

Whether or not the fountain grass individuals with a FS genotype are superior in gametophytic development or have a higher level of fitness in direct response to the enzyme produced by this PGI heterodimer remains unknown.

The S allele may be somehow linked or associated with a developmental disadvantage in the dwarf growth habit since low numbers of these individuals were detected. Dwarfism in pearl millet was reported as the result of the inability to use gibberellic acid, GA_3 (Krishna Rao, et.al.,1981).

The appearance of FS individuals from SS parents in the dwarf genotype appears to be the result of outcrosses, although every attempt was made to bag heads prior to any stigma emergence, and all bags appeared to be secure.

In conclusion, fountain grass expressed polymorphism at only one locus studied, PGI-2. The inheritance for three generations at this locus followed typical Mendelian segregation. The SS band appears to be an isozyme marker for the dwarf growth habit, since it appeared in all of the 30 phenotypically dwarf plants tested. Further analysis with only plants, not seeds which are sacrificed, is necessary to determine if linkage is involved between the genes controlling dwarf growth habit and the PGI-2 locus in fountain grass.

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Table 1. Segregation and X^2 statistic for inheritance of phosphoglucoisomerase (PGI-2) in four growth habits of fountain grass.

Cross	Progeny Segregation FF	FS	SS	Test Ratio	X^2	P
p[z], selfed						
FF						
F_1	180	-	-	--	--	--
F_2	23	-	-	--	--	--
F_3[y]	0			--	--	--
p x u						
FF x FS						
F_1	14	13	-	1:1	0.04	.85
F_2	24	1	-	--	--	--
F_3[y]	1	1	-	1:2:1	1.50	.47
p x m						
FF x FF						
F_1	27	-	-	--	--	--
F_2	32	-	-	--	--	--
F_3	26	-	-	--	--	--
p x d						
FF x SS						
F_1	-	31	-	--	--	--
F_2	11	26	10	1:2:1	0.51	.77
F_3	11	34	10	1:2:1	1.95	.38
u, selfed						
FS						
F_1	14	17	10	1:2:1	1.96	.38
F_2	13	39	11	1:2:1	3.69	.16
F_3	9	13	6	1:2:1	0.78	.68
u x p						
FS x FF						
F_1	22	20	-	1:1	0.09	.75
F_2	7	24	8	1:2:1	2.11	.35
F_3	25	-	-	--	--	--
u x m						
FS x FF						
F_1	15	15	-	1:1	0.00	.99
F_2	26	-	-	--	--	--
F_3	30	-	-	--	--	--
u x d						
FS x SS						
F_1	1[x]	13	16	1:1	1.33	.57
F_2	6	19	8	1:2:1	0.99	.61
F_3	9	17	7	1:2:1	0.27	.87

[z] p = prostrate, u = upright, m = mound, d = dwarf.
[y] little or no seed set
[x] self

Table 1. Segregation and X^2 statistic for inheritance of phosphoglucoisomerase (PGI-2) in four growth habits of fountain grass.

Cross	Progeny Segregation FF	FS	SS	Test Ratio	X^2	P
m[z] selfed						
FF						
F_1	38	-	-	--	--	--
F_2	26	-	-	--	--	--
F_3[y]	3	6	3	--	--	--
m x u						
FF x FS						
F_1	24	26	-	1:1	0.08	.77
F_2	37	-	-	--	--	--
F_3	25	-	-	--	--	--
m x p						
FF x FF						
F_1	25	-	-	--	--	--
F_2	25	-	-	--	--	--
F_3	10	-	-	--	--	--
m x d						
FF x SS						
F_1	11[x]	19	-	--	--	--
F_2	13	27	6	1:2:1	3.49	.18
F_3	25	-	-	--	--	--
dwarf,selfed						
SS						
F_1	-	-	29	--	--	--
F_2	-	6	51	--	--	--
F_2leaf samples		5	30	--	--	--
F_3	6 seed[y]					
d x u						
SS x FS						
F_1	-	28	22	1:1	0.72	.40
F_2	9	25	6	1:2:1	2.95	.23
F_3[y]	3	7	3	1:2:1	0.07	.96
d x p						
SS x FF						
F_1	-	29	-	--	--	--
F_2	13	23	5	1:2:1	3.64	.16
F_3	13	28	9	1:2:1	1.36	.51
d x m						
SS x FF						
F_1	-	27	-	--	--	--
F_2	15	28	7	1:2:1	3.28	.19
F_3[y]	10	-		--	--	--

[z] p = prostrate, u = upright, m = mound, d = dwarf.
[y] little or no seed set
[x] selfs

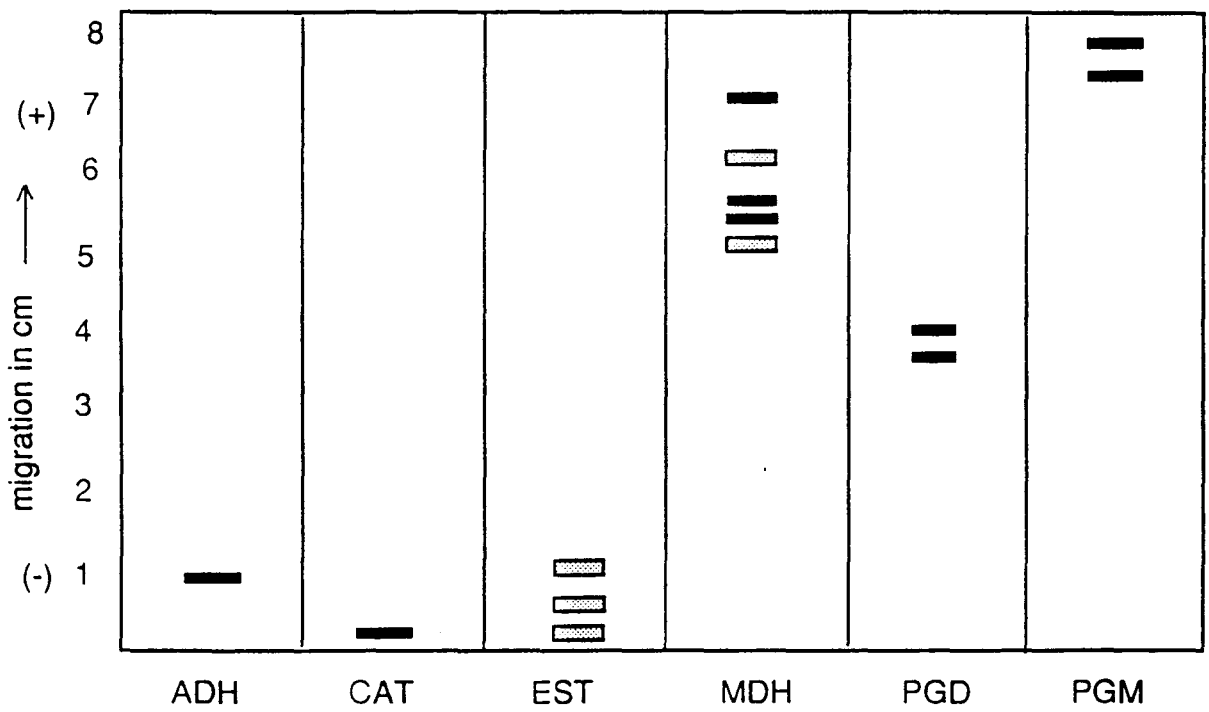

Figure 1. Interpretative drawing of enzyme systems that did not show variation in banding patterns among tested fountain growth habits.

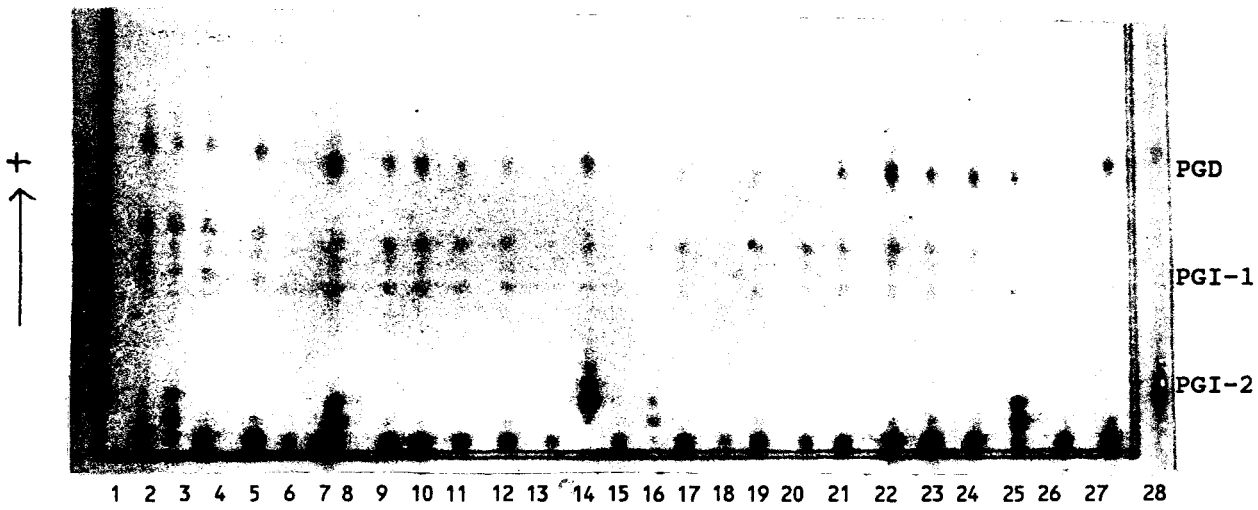

Figure 2. PGI expression in fountain grass. PGI-1 and PGD were monomorphic, PGI-1 expression was weak. PGI-2 was expressed as one fast band, FF, one slow band SS, or as a triple band, FS. Lanes 1,14,28 are controls, dry seed from the prostrate plant, expressing FF alleles; the other 25 lanes are extracts from young leaf tissue of F_2 plants putative selfs from a dwarf parent. Plants with the FS alleles in lanes 3,8,16, and 25 were not phenotypic dwarfs, all other 21 lanes show the expected SS alleles and were dwarfs.

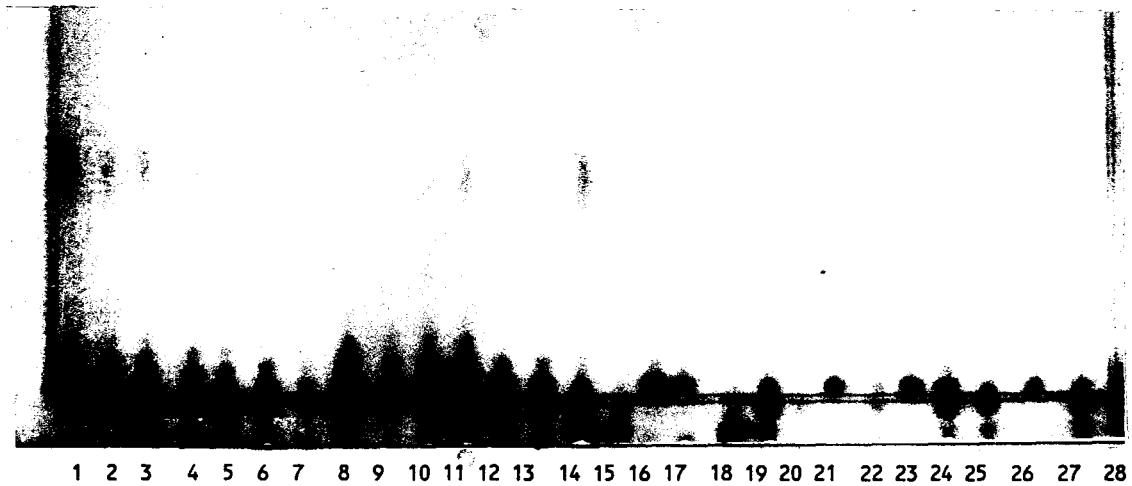

Figure 3. Inheritance of PGI-2 in two generations of fountain grass. Lanes 1 and 2 show extracts from seeds of the female parent, a prostrate plant with FF alleles;lanes 14 and 15 are the extracts from seeds of the male parent, a dwarf plant with SS alleles; lanes 3-13 show extracts from seeds of the F_1 generation, all exhibiting FS alleles; lanes 16-28 show segregation of 5 FF, 5 FS and 1 SS individuals, randomly selected seeds from the F_2 generation.

For Hort Science

INHERITANCE OF GROWTH HABIT IN FOUNTAIN GRASS[1]

M. Hockenberry Meyer, D. B. White and P. D. Ascher[2]

Dept. of Horticultural Science
University of MN, St. Paul MN 55108

ABSTRACT

Four genetically controlled growth habits: dwarf (d); upright (u); mound (m); and prostrate (p); were identified in fountain grass, Pennisetum alopecuroides (L.) Spreng., and crossed in a complete diallel in 1990 and 1991. The dwarf and upright forms appear to share similar genetic backgrounds, as do the mound and prostrate habits. The dwarf form appears to be controlled by one recessive gene. The upright form appears to be heterozygous for this dwarf gene, since it produced dwarf progeny when selfed. Progeny from four crosses, d x p; p x d; m x d; and d x m, exhibited heterosis in culm length, exceeding either parent. Upright and mound appear to be dominant traits, but the prostrate appears to be recessive and may involve epistasis.

Fountain grass is typically a bunch type perennial grass with numerous culms 90-125 cm tall with purple or mauve spike-like inflorescences. It has been grown as an ornamental in the U.S. since the early 1940's (Bailey, 1949). However, only one form is readily available in the trade (Foerster, 1982; Meyer, 1975).

Floras from Japan (Ohwi, 1965; Okuyama, 1982), Burma (Bor, 1960), China (Steward, 1958;ICS, 1976), the Okinawa Islands (Walker, 1976); and Australia (Black, 1978; Cunningham, 1981) list fountain grass as a native species. Its natural range extends from 48° N latitude in Manchuria (Kitagawa, 1979) to 38° S latitude, along the coast of New

[1] Paper No._____, of the Scientific Journal Series, University of Minnesota Agricultural Experiment Station.
[2] graduate student and professors, respectively.

South Wales in Australia (Wheeler, 1982). Habitat varies from coastal areas (Cunningham, 1981), wet lowland (Bor, 1960; Ohwi, 1965; Black, 1978), and open plains (Bor, 1965) to inland mountains (Kitagawa, 1979). Indeed, with its wide range of adaptation, other forms may be expected to exist. No reports of breeding improvement or basic genetics of fountain grass could be found in the literature except the chromosome number 2n = 18 (Ono, 1953). In this report, we describe four growth habits of fountain grass and a possible basis for the genetics and inheritance of these growth habits.

METHODS AND MATERIALS

Fountain grass was acquired from nine sources as seed or plants and evaluated for morphological variation. Material received as plants was grown in a Waukegan silt loam soil in St. Paul, MN or in a Hayden loam soil at the University of Minnesota Landscape Arboretum in Chanhassen, MN. Seeds were germinated in the greenhouse and the seedlings transplanted to field locations as indicated. Plants were spaced 1m apart and normal cultural practices were maintained such as watering, weed control, and fertilization.

Morphological characters measured for variation were culm length, culm angle, leaf length and width, inflorescence length, flowering date, peduncle length, internode length, flowering culms per plant, leaf and seed color (based on the Royal Horticulture Society's Colour Chart), spikelets/cm, and seed weight (gm/100seed). Based on variation in these characters, four growth habits were identified, Table 1, and crossed in a complete diallel.

With few exceptions, fountain grass is almost exclusively protogynous, stigmas precede anther exertion and dehiscence by 2 to 3 days (Meyer,unpublished data), which is a natural means of assisting cross pollination (Burton,1974) and obtaining hybrids (Burton,1991). Inflorescences on parents selected for stable differences in growth

habits were bagged prior to stigma emergence, sometimes when the inflorescence was still partially in the boot. For crosses, the appropriate pollen was applied when receptive stigmas were observed, and the heads were immediately rebagged. Visual identification of distinct hybrids in the F_1 generation offered sufficient evidence that minimal selfing occurred. Inflorescences were collected approximately one month after pollination, the seed was hand cleaned and weighed.

In 1990, two crosses were made to half sibs of the male parents, because the designated parent plants had insufficient pollen. In 1991, two crosses were made with F_1 progeny as parents, again due to the lack of available pollen, thus these segregation ratios are similar to F_2's.

In March 91 and 92 at least 50 randomly selected seeds per F_1 line were germinated on blotter paper and then transplanted to cell packs in the greenhouse. In late May seedlings were field planted in a Waukegan silt loam in a 1m x 1m randomized design by lines. Normal cultural practices were maintained such as weeding, irrigation and fertilization. In 1991, F_2 seeds were obtained by bagging at least 5 random heads per F_1 line prior to stigma emergence. Inflorescences were collected, seeds were cleaned and germinated, and subsequent plants grown as outlined above.

A total of 1368 F_1 and F_2 progenies were grown for field evaluation and classification by growth habit.

Morphological characters were measured in late August, for each F_1 and F_2 family. Culm angle was measured by placing a 1-m dia. protractor adjacent to each plant and estimating the average and minimum culm angle relative to the soil surface 0°, (i.e. the ground angle). All F_1 and F_2 plants were categorized by growth habit based on culm angle, culm length, leaf width and leaf length. Segregation ratios for the two generations were analyzed for goodness-of-fit to test ratios.

RESULTS

Four growth habits, prostrate, upright, mound, and dwarf, were

identified based on the morphological variation over two years of evaluation, (Table 1 and Figure 1).

The upright form is characterized by long, medium textured foliage, tall upright (>60°) culms, with long, pendulous inflorescences well above the foliage. The mound form has slightly shorter leaves, which are quite coarse, the inflorescences are on shorter, stiff culms, just above the foliage; culm angle is typically 50 to 60°. The prostrate habit is distinguished by short, coarse leaves and stiff culms quite low to the ground, and culm angles of 20 to 50°, and stiff inflorescences just above the foliage. The dwarf has very fine textured foliage and flexible pendulous inflorescences well above the foliage; most culms are at a 60° angle or greater.

The dwarf selfs were all dwarfs and resembled the parent in 1991 (Table 2). In 1992 only 13 seeds were obtained from dwarf selfs and when grown these were larger plants with coarse textured foliage. Isozyme analysis (Meyer and White, 1993) identified these plants as non-dwarfs, probably outcrosses, not selfs. The F_2 generation of dwarf selfs resulted in 26 dwarfs,4 very small, < 25 cm, plants and 5 large coarse plants which isozyme analysis also showed to be nondwarf.

The upright selfed produced only three progeny (all uprights) in 1990 but in 1991 it produced a ratio of three upright to one dwarf (Table 2). The F_2 (obtained from selfing an upright) produced only upright plants.

The u x d crosses and reciprocals resulted in both upright and dwarfs in the F_1 in 1991 and 1992. In 1991, an F_1 upright plant from both families (u x d and d x u) was selfed to obtain the F_2 which resulted in 11 upright and 15 dwarfs for the u x d cross and a ratio of 3 small uprights (really intermediates) to one dwarf in the d x u cross.

The p x d and m x d crosses and reciprocals resulted in F_1 hybrids which expressed heterosis, as culm lengths were greater than either parent (Table 3). The F_2's of the p x d and d x p crosses segregated

into a ratio of 14 intermediates (plants with some characteristics of either parent) to 1 prostrate and 1 dwarf (Table 4). The F_2's of the m x d and d x m crosses segregated into a ratio of 9 mound to 6 intermediate (plants with some characteristics of either parent) to 1 dwarf. One cross, p x d when repeated in 1992, was made with F_1 plants because of lack of pollen from the parents and showed an unusual segregation 5 mound to 1 prostrate.

The u x m and u x p crosses and reciprocals resulted in F_1 plants that were not like either parent (Table 5). These plants were about as tall as the upright, with open spreading culms (culm angles ranging from 20^0 to 90^0), with medium textured foliage. Thus these hybrids did not fit any growth habit of the parental types. F_2 lines with mound and upright as original parents were all mounds (Table 5). F_2's from p x u and u x p crosses segregated into 12 or 15 intermediates (plants with characteristics of both parents) and 3 or 1 prostrate, respectively.

The mound selfs were all mounds in 1991, but segregated to 3 mound, to 1 prostrate or very small, < 25 cm progeny, in 1992 (Table 6). F_2 plants segregated into 3 mound to 1 prostrate. The F_1 m x p and reciprocal crosses were all mounds except 2 prostrate progeny. The F_2 segregated to a ratio of 12 mound, 3 prostrate and 1 very small, < 25 cm, plant.

The selfed prostrate resulted in unusual ratios of mound, prostrate and very small, < 25 cm, progeny (Table 6).

DISCUSSION

The dwarf growth habit reported here appears to be controlled by one recessive gene, designated as dd, since F_1 and F_2 selfs were almost all dwarfs (Table 4).

The upright appears to be heterozygous for this gene controlling height, or Dd, because the upright selfs resulted in both dwarf and upright plants. Progeny from the u x d cross fit a 2:1 segregation ratio instead of the expected 1:1, perhaps due to uncovering lethal recessive

alleles that were shared with these two growth habits or perhaps the upright has greater vigor or possesses a competitive advantage over the dwarf.

Crosses between d x m or d x p and reciprocals resulted in all nondwarf plants. The fact that these crosses (d x m; d x p; p x d; m x d) resulted in large heterotic progeny (Table 2) exceeding either parent in mean culm length, may indicate that the dwarf growth habit differs in genetic background from the prostrate or mound. This idea is further supported by the different sources of origin for the three types (Table 1).

Crosses involving the u x m or u x p and reciprocals resulted in F_1 hybrids that resembled none of the growth habits and may also be the result of different genetic backgrounds.

Based on these crosses, the mound and prostrate appear to have a different allele for the height or growth habit gene, and may be tentatively designated as XX.

Isozyme analysis further supports these differences in genetic backgrounds. Meyer and White (1993), reported that the mound and prostrate growth habits have common isozyme alleles, labeled FF, at the phosphoglucoisomerase-2 locus while the dwarf exhibited a different allele, SS, and the upright had the heterozygote or FS alleles at this locus.

Selfs and crosses between the prostrate and mound growth habits did not fit any expected or normal segregation ratios. The mound appears to be dominant over prostrate because prostrate plants were always in the minority, even from prostrate selfs. These two growth habits may be the result of a set of different genes that are functioning completely independently of the gene controlling height in the dwarf and upright growth habits. Because the mound and prostrate were selections from one seed lot (Table 1), they may be exhibiting hybrid breakdown, or inbreeding depression which further complicates the inheritance analysis

of this report.

More than one gene appears to regulate growth habit in the mound and prostrate. Because of the unusual ratios of prostrate plants epistasis as well as recessive genes may be involved in controlling this growth habit.

Thirty-seven or 3% of the progeny were very small plants, < 25 cm, and may be the result of inbreeding depression or the accumulation of recessive alleles that resulted from selfing. These plants occurred in several families, with all four growth habits as parents. With one exception, no very small plants occurred in the progeny of crosses involving the proposed different genetic backgrounds. These very small plants rarely flowered, were prone to leaf spot diseases and sometimes died prematurely. The protogynous flowers of fountain grass seem to be evidence that outcrossing is favored over self pollination. Two generations of selfing and crossing closely related individuals appeared to result in higher levels of very small progeny and distorted segregation ratios.

Several questions still remain concerning the inheritance of these growth habits, however, some conclusions can be drawn from this research. First, the dwarf and upright appear to share similar genetic backgrounds and the dwarf form is recessive to the upright. Secondly, the mound and prostrate also share similar backgrounds, which are different from the the dwarf and upright, and the prostrate is recessive to the mound. And thirdly, crosses between different backgrounds resulted in heterosis whereas another phenomena, inbreedling depression, appeared to occur if plants with similar backgrounds were crossed or selfed.

Growth habit can be a complex trait influenced by the environment as well as genotype. However, it is possible that 1 or 2 genes can have a major influence on plant form (for example, genes that play a major role in the gibberellic acid pathway). Thus one gene could have a

cascade effect that results in morphological changes resulting in the growth habits described in this paper.

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TABLE 1. Characteristics of parents of four selected growth habits in fountain grass.

	Culm lgth. (cm)	Leaf lgth. (cm)	Leaf wdth. (cm)	Fl lgth. (cm)	Culm angle°	S[y]
u[z]	121	73	.7	14	75	KB
m	89	58	1	11	60	GL
p	79	32	.8	11	45	GL
d	72	31	.4	9	68	JG

[z] u = upright; m = mound; p = prostrate; d = dwarf.

[y] KB, Kurt Bluemel Nursery, MD. labeled as Pennisetum alopecuroides. GL, USDA Germplasm Lab,Experiment,GA, origin Korea; JG, John Greenlee Nursery,Pomona,CA, labeled as Pennisetum alopecuroides 'Weserbergland'.

TABLE 2. Growth habit segregation ratios of F_1 and F_2 progeny from dwarf and upright selfs and crosses in fountain grass.

cross	n	observed	expected	X^2	P
dwarf selfed					
F_1[z]	25	25d[y]	all d	-	-
F_1R	13	13 non-d	all d	-	-
F_2	35	26d:4idb:5non-d	all d	16	.00
u selfed					
F_1	3	3u	3u	-	-
F_1R	41	33u:7d	3:1	1.03	.31
F_2	9	9u	all u	-	-
u x d					
F_1	29	29u:9d	1:1	4.2	.04
F_1R	17	11u:5d+1ibd	1:1	1.5	.22
F_2	26	11u:15d	3:1	8.5	.00
d x u					
F_1[x]	2	1u:1d	1:1	-	-
F_1R[x]	8	1u:6d+1ibd	1:1	3.7	.06
F_2	42	33I:9d	3:1	.23	.63

[z] F_1 1991; F_1 R = repeated F_1 in 1992.
[y] d = dwarf; u = upright; I = intermediate, midway between parents; ibd = inbreeding depression.
[x] ♂ parent is ½ sib.

TABLE 3. F_1 Mean culm length (cm) of four crosses as compared to parents in fountain grass.

Cross	Parents	F_1 1991	F_1 1992
p x d[z]	79;72	107**	115**
d x p	72;79	98**	110**
m x d	89;72	93*	98**
d x m	72;89	85	110**

[z] p = prostrate; u = upright; m = mound; d = dwarf.

*, ** Significantly different from either parent at P ≤ 0.05 or 0.01, respectively.

TABLE 4. Growth habit segregation ratios of F_1 and F_2 progeny from crosses between dwarf and mound or prostrate growth habits in fountain grass.

cross	n	observed
prostrate x dwarf		
F_1[z]	37	37H[y]
F_1R[x]	42	35m:7p
F_2	45	39I:4p:4d
dwarf x prostrate		
F_1[w]	31	31H
F_1R[x]	6	6 H
F_2	43	37I:2p:4d
mound x dwarf		
F_1	20	20H
F_1R	46	46H
F_2	31	17m:11I:3d
dwarf x mound		
F_1	32	32H
F_1R	8	8H
F_2	40	24m:12I:4d

[z] F_1 1991; F_1 R = repeated F_1 in 1992.
[y] H = Hybrid with heterosis; d = dwarf; p = prostrate; m = mound;I = intermediate, midway between parents.
[x] F_1 generation used as parents.
[w] ♂ parent is ½ sib.

TABLE 5. Growth habit segregation ratios of F_1 and F_2 progeny from upright and mound or prostrate crosses in fountain grass.

cross	n	observed
upright x mound		
F_1[z]	25	25 h[y]
F_1R	43	43 h
F_2	39	39 m
upright x prostrate		
F_1	24	24h
F_1R	41	41h
F_2	50	48I:2p
mound x upright		
F_1	25	25 h
F_1R	44	44 h
F_2	39	39 m
prostrate x upright		
F_1	19	19h
F_1R	33	33h
F_2	45	33I:11p:1ibd

[z] F_1 1991; F_1 R = repeated F1 in 1992.
[y] h = hybrid, not like either parent; p = prostrate; m = mound; I = intermediate, midway between parents; ibd = inbreeding depression;

TABLE 6. Growth habit segregation ratios of F_1 and F_2 progeny from mound and prostrate selfs and crosses in fountain grass.

cross	n	observed
mound selfed		
F_1[z]	37	37m[y]
F_1R	43	30m:5p:8ibd
F_2	12	10m:2p
mound x prostrate		
F_1	2	2m
F_1R[x]	13	11m:2p
F_2	36	26m:3p:7ibd
prostrate x mound		
F_1	37	37m
F_1R	27	27m
F_2	43	35m:2p:6ibd
prostrate selfed		
F_1	23	16m:6p:1ibd
F_1R	34	22m:10p:2ibd
F_2	21	11m:4p:6ibd

[z] F_1 1991; F_1R = repeated F_1 in 1992.
[y] m = mound; p = prostrate; ibd = inbreeding depression.
[x] F_1 generation used as parents.

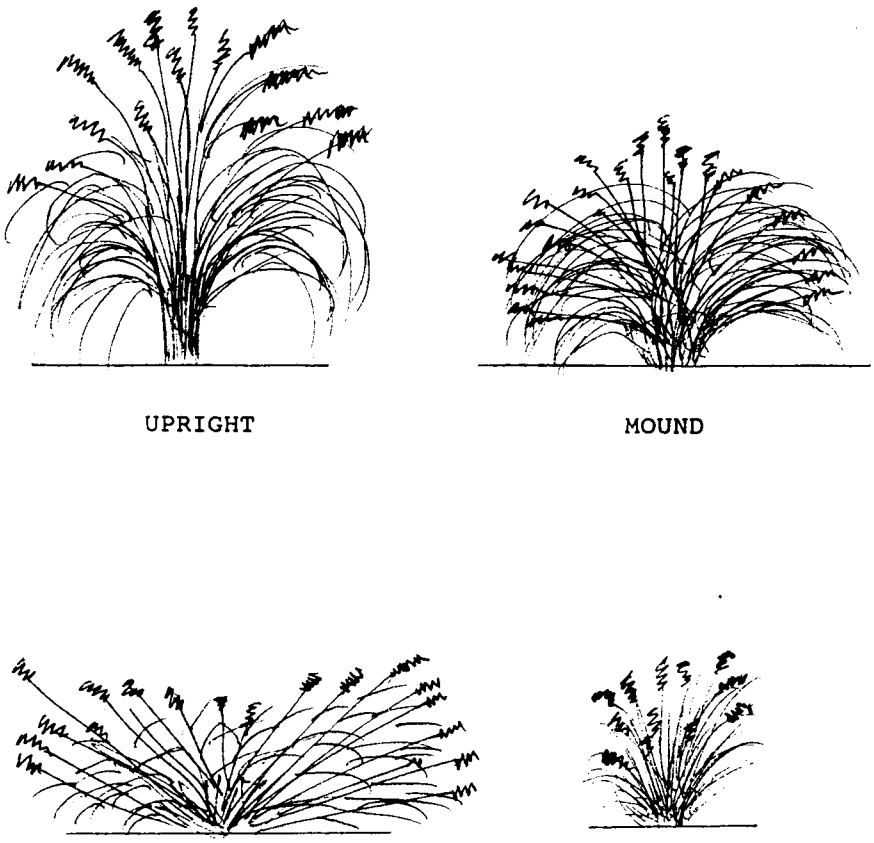

FIGURE 1. FOUR GROWTH HABITS OF FOUNTAIN GRASS.SCALE: 1cm = 30cm.

For Hort Science

IN VITRO POLLEN GERMINATION IN FOUNTAIN GRASS[1]

M. Hockenberry Meyer, D. B. White[2]

Dept. of Horticultural Science
University of Minnesota, St. Paul, MN 55108

ABSTRACT

Germination of pollen from plants in two generations (parents and F_1 selfs) of four growth habits, prostrate, upright, mound and dwarf, of fountain grass, Pennisetum alopecuroides (L.) Spreng., was investigated in vitro with a 25 g kg sucrose and 100 mg ml boric acid solution. Germination ranged from a low of 4% in the mound F_1 to 47% in the upright parent which was significantly more than from plants of the other three growth habits and two of the F_1's. Wide variation in germination was found within and between samples.

Fountain grass is a bunch type ornamental grass that has been grown in the U.S. for many years (Bailey,1949;Meyer,1975). Except the chromosome number, 2n = 18 (Ono, 1953), very little information is available concerning its breeding behavior. To our knowledge, there has been no effort to develop new forms or cultivars. In 1987, a breeding program for fountain grass was initiated at the University of Minnesota. Preliminary research revealed highly variable seed set in self, open and cross pollinations from most accessions both in the field and greenhouse.

Fountain grass is protogynous, (Meyer, unpublished data) which favors outcrossing (Burton,1974). Although variation in seed set could be due to several factors, pollen viability was identified as one

[1] Paper No._____, of the Scientific Journal Series, University of Minnesota Agricultural Experiment Station.
[2] graduate student and professor, respectively.

of the most critical and easiest to test.

Pollen viability in the Poaceae and especially Pennisetum has been estimated by using vital stains (Simpson and Bashaw, 1969), in vitro germination and in vivo tube growth (Helsop-Harrison et.al 1982,1984), and seed set (Cooper and Burton 1965). Although the ultimate functional test ought to be fertilization as measured by seed set (Helsop-Harrison et.al. 1984), incompatibility reactions and other phenomena may interfere with pollen function (Stanley and Linskens,1974) and seed set involves considerable time and effort.

Vasil (1960) first reported in vitro germination of Pennisetum glaucum (L.)R.Br., pearl millet. Hill (1991) found in vitro germination of pearl millet and other species of Pennisetum to be preferred over iodine and fluorescein diacetate (FDA) screening methods. Abdul-Baki (1992) reported good correlation between in vitro germination and fluorescing tomato pollen in an FDA solution. Both Hill and Abdul-Baki indicated some difficulty in scoring FDA due to the variation in intensity of the florescence. In vitro germination is considered the standard and although it may show false negatives, it is simpler than seed set correlation (Heslop-Harrison,1984).

The objective of this research was to determine 1) if fountain grass pollen could be germinated in vitro 2) whether germination differed between accessions and 3) if viability decreased with selfing.

METHODS AND MATERIALS

Evaluation of fountain grass germplasm from several sources (Table 1) resulted in the identification of four growth habits: 1) an upright form which is commonly found in the trade, 2) a dwarf, not as common, but also found in the trade, 3) a mound and 4) a prostrate. The latter two plants were selected from a seed lot from the USDA Germplasm Lab in Experiment, GA. These four growth habits and their F_1 progeny were the source of pollen for this investigation. Detailed descriptions of these forms and their inheritance as well as isozyme analysis have been

reported (Meyer and White,1993; Meyer et.al.1993). habit was distinguished by several morphological characteri culm angle in relation to (horizontal) ground level, culm heigh h, leaf length and flower length. Four plants, selected as typi growth habit were selected and grown in a Hayden loam soil at th University of Minnesota Landscape Arboretum.

Pollen was collected from individual plants or F_1 populations approximately twice weekly from 1000 to 1200 hours by shaking inflorescences at anthesis into glassine pollen bags. Collections were made from early August until mid September 1992. Immediately after collection, the pollen was taken to the lab. Occasionally, if dehiscence was delayed due to cool, wet weather, entire heads were taken to the lab where the culms were placed in distilled water until the anthers dehisced.

Once in the lab, pollen was placed on a glass slide in a covered petri dish with filter paper moistened with distilled water. This hydration treatment was necessary for germination and required 20 to 30 minutes exposure. Pollen was then dusted onto approximately 0.01 ml of solution and sealed in a depression slide at room temperature. The germination solution was 25 g kg sucrose and 100 mg ml boric acid in distilled water. Lower concentrations of sucrose or boric acid and the elimination of hydration resulted in poor germination. Very fresh pollen also appeared to be critical for success. Preliminary data (Meyer,1990) indicated that viability deteriorated in as little time as one hour.

Germination counts were made within 24 hours. A pollen grain was considered to be germinated when the length of the pollen tube was equal to or longer than the diameter of the grain. A minimum of 300 grains per slide were counted. A pollen grain that germinated was considered to be viable.

RESULTS AND DISCUSSION

Of the four parents, the upright growth habit had significantly

more viable pollen, 47% (Table 2). A half sib of this plant produced pollen of similar viablity, 44%. The mound form produced 22% viable pollen, the dwarf 12% and the prostrate exhibited the lowest viability, 8%.

In the F_1 generation, or selfs, two of the progeny exhibited a decrease in viability, the upright and the mound, to 25% and 4% respectively. However, the prostrate and the dwarf increased in viability with 17% and 27% respectively. Although the F_1 mound plants were sampled more than 23 times, the pollen viability was never above 10%. This was significantly less than mound parent, the upright parent and F_1, and the dwarf F_1. Although not statistically significant, biologically, the F_1 mound pollen appears to be a poor choice for use in crosses, and a large amount of it may be necessary for seed set.

Wide variation within samples was found in the parent as well as the F_1 generation. This variation may be due to several factors, such as pollen age, environmental effects of temperature (low or high), seasonal variation, inbreeding depression, or sampling variation.

In conclusion, in vitro germination can be used to determine pollen viability of fountain grass. Different growth habits expressed different pollen viability. In this research, the upright parent produced significantly more viable pollen than plants of the three other growth habits. After one generation of selfing, the F_1 mound pollen never exceeded 10%, and had the lowest mean of 4%. Fountain grass appears to have sufficient pollen viability to be used in a breeding program, although wide variation was found within as well as between samples.

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TABLE 1. Source of four selected growth habits in fountain grass.

Description	Source
upright	Kurt Bluemel Nursery, Baldwin, MD
mound	USDA Germplasm Lab Experiment, GA, origin: Korea
prostrate	USDA Germplasm Lab Experiment, GA, origin: Korea
dwarf	John Greenlee Nursery, Pomona, CA

Table 2. Pollen Germination of Four Growth Habits and F_1 Progeny in fountain grass.

Growth Habit	#slides[z]	Germination % Mean	Range
prostrate	14	8 ab[y]	0-29
F_1	11	17 ab	.1-28
upright	13	47 c	18-61
upright(½sibs)	18	44 bc	3-77
F_1	17	25 bc	6-58
mound	15	22 b	3-64
F_1	23	4 a	.2-10
dwarf	16	12 ab	1-28
F_1	14	27 bc	3-66

[z] each slide represents a minimum count of 300 grains,100 grains/field, 3 fields per slide.

[y] Means followed by different letters are significantly different at P = 0.05, as determined by LSD Test after square root transformation.

GENERAL DISCUSSION

Objectives of this research were to 1) determine if growth habit inheritance fit a one or two gene model, 2) assess isozyme variation in four growth habits as well as isozyme inheritance and 3) to determine if pollen viability varied between growth habits and F_1 progeny.

With some exceptions, dwarf and upright growth habit inheritance could fit a one gene model with the dwarf as a homozygous recessive trait, dd, and the upright as a heterozygote, Dd or homozygote DD. The dwarf form appeared to be the easiest to explain because it usually was true breeding and was not recovered in the F_1 progeny of crosses with the prostrate or mound forms. The fact that heterotic progeny resulted from these crosses seemed good evidence that the dwarf growth habit had a different genetic background than the mound or prostrate.

The prostrate growth habit was the most difficult to explain and may involve epistasis, or completely different genes than the one controlling the dwarf growth habit. Not only did the prostrate form not breed true into the second generation, but the majority of the progeny were mounds, the prostrate form was always in the minority.

Perhaps future research on growth habit should center on one form, such as the prostrate, and analyze families of progeny resulting from crosses using several different prostrate parents with other growth habits. Using only one individual prostrate plant may have limited this study.

The appearance of non-dwarf progeny from putative selfs of the dwarf was not expected. Outcrossing seems to be the only explanation for the origin of these plants. Although every effort was made to bag the heads prior to stigma emergence, the dwarf form was very protogynous and heads half in the boot almost always had visible stigmas.

Isozyme analysis further supported that these non-dwarf plants were not, at least as far as PGI-2 variation is concerned, the same genotype as the dwarf.

It is interesting to speculate on the impact of the PGI-2 gene product and its direct impact on the dwarf growth habit. Is it possible that this isozyme, the SS or homozygous slow allele, may be a key protein in regulating the dwarf phenotype ?

The direct impact on plant physiology as a result of a specific enzyme, or a combination of isozymes has been discussed by Mitton (1989). Brown and Munday (1976) and Rainey et.al. (1987) reported that enzyme polymorphisms and specific enzyme genotypes demonstrate physiological differences in bromegrass and ryegrass respectively. McNaughton (1974) reported on malate dehydrogenase, MDH, variation in Typha latifolia. He stated that phenotypic differences on which the environment imposes selection must come from metabolic differences. In this case, plants originating from different environments exhibited different properties of MDH such as thermal stability and activation energy.

Watt (1983) reported that Colais butterflies with PGI heterozygous genotypes were "kinetically favored" and females preferentially mated with these genotypes.

The alcohol dehydrogenase-1 locus in corn has been studied extensively (Schawartz and Laughner,1969) as an example of a superior heterozygote that can be directly related to protein variation. Two common alleles segregate as homodimers (one is unstable but highly active, the other stable but has a low level of activity). However in crosses, they combine to form a heterodimer that has the biochemical advantage of being both stable (temperature, pH resistant) and highly active. This is an example of a superior heterozygote that can be related directly to protein variation.

Further studies are needed between the genes regulating the dwarf growth habit and the PGI-2 locus in fountain grass to determine what the physiological impact is of the SS alleles.

Analysis of all F_1 and F_2 dwarf phenotypes using plants, not seeds,

and the expression of alleles at the PGI-2 locus is needed to confirm the linkage of genes controlling PGI-2 and the dwarf growth habit.

Acquiring a diversity of fountain grass germplasm was difficult although 9 sources are listed in Appendix 1. It is not known how diverse these germplasm samples really are. Although the popularity of fountain grass had increased in the last 10 years, as recently as 1985, only five wholesale nurseries sold this plant. Kurt Bluemel, Baldwin, MD, has been the main wholesale supplier for the rest of the U.S. Since fountain grass is almost always vegetatively propagated, it is possible that the majority of the germplasm in this country is quite similar.

Efforts to receive germplasm directly from other countries such as China, Japan, Burma, Australia, etc. through the USDA were not successful. Other than making a personal trip to collect germplasm, it is difficult to acquire a diversity of germplasm for a plant such as this, where little research work has been done.

Isozyme analysis is often used as a measure of germplasm diversity and in this case polymorphism was found only at one locus of the seven systems that were resolved. Weeden and Wendel (1989) concluded that "a minimum of 30-40 polymorphic isozyme loci" should be found in species with reasonable genetic diversity. 70% of the 70 or so loci with observable products have been found to be polymorphic in maize and garden pea, two species studied extensively.

From a growth habit standpoint, the genetics seemed varied and complex, yet from the isozyme analysis, the amount of variation was low. And of the 1000's of genes operating in fountain grass only a minute fraction were studied.

Pollen viability as measured by in vitro germination varied greatly. Although the technique was successful, the results varied from day to day both in the parents and F_1 progeny. Environmental conditions and a short life span may have contributed to the wide fluctuation in results. Pollen viability was significantly higher for the upright

growth habit. Although there was variation in the F_1 generation the mound F_1 pollen had a mean of only 4%, which could make it a poor choice in a breeding program.

Several appendices are included that offer supporting data in seed germination and seed set. In addition, preliminary pollen germination and cold hardiness data are also included.

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APPENDIX 1

Table 1. Sources of germplasm Pennisetum alopecuroides (L.) Spreng.

Date	Number	Source[z]	Parent[y]
7/87	UM 1805	Kurt Bluemel,Inc. 2740 Greene Lane Baldwin,MD 21013-9523	upright
7/87	UM 1803	Greenlee Nursery 301 E.Franklin Ave. Pomona,CA 91766	dwarf
7/87	UM 9163	Bluemount Nursery 2103 Blue Mount Road Monkton, MD 21111	
4/88	90190	USDA Germplasm Lab, Experiment,GA	mound prostrate
7/88	9091 413373 413795	Jack Murray,USDA,National Turfgrass Evaluation Program,Beltsville,MD	tawny mound
9/88	UM 9166	MN Landscape Arboretum Seed from perennial garden; 1985 acquisition from Spring Hill Nursery Ohio	
11/89	UM 1807	Cornell University Ithaca, NY 14853	
2/90	UM 9167 UM 9168	China and Japan collected by W.W.Hanna Coastal Plains Experiment Station, Tifton,GA	

[z] Cornell Univ.,Landscape Arboretum and the Germplasm Lab material was received as seed, all other sources were plants.

[y] upright parent was selected from open pollinated progeny resulting from seed collected from plants received from K Bluemel labeled P.alpecuroides; mound and prostrate growth habits came from seed collected from eight open pollinated plants from the Germplasm Lab seed; dwarf parent was selected from progeny of a selfed dwarf plant labeled Pennisetum alopecuroides 'Weserbergland' from Greenlee Nursery.

APPENDIX 2

MORPHOLOGICAL VARIATION BETWEEN GROWTH HABITS IN FOUNTAIN GRASS

Thirteen morphological characters were measured for variation between four growth habits and the F_1 generation in Fountain Grass. All characters were measured in 1991, however only four (culm length, leaf length, leaf width, culm angle) were remeasured in 1992, since these were the most definitive in determining growth habit. All figures represent means, except the data on seed weights, flower and leaf color, and days to flowering.

Table 1. Morphological variation of growth habits in Fountain Grass.

Plant	CL[z] (cm)	IL (cm) 3rd	2nd	last	P (cm)	FL (cm)
upright						
64-20	121	8	9.3	14	58	14
F_1	94	7	9	17.5	66	14
64-3 ½s[y]	117	8.5	12.5	15.5	62	14
F_1	93	--	--	12.5	68	13
mound						
35-9	89	8.3	11.8	11	50	11
F_1	60	—	6	8.5	41	10
35-20fs[y]	89	8.8	10.9	11.3	50	11
F_1	65	--	7.5	9.3	49	.9
prostrate						
14-2	79	5	8	10	46	11
F_1	80	--	8.2	12	56	12
22-13ap[y]	88	--	--	--	54	10
23-9 ap[y]	79	5	7.3	9.8	42	12
F_1	76	--	8.6	14.4	48	12
dwarf						
9094	72	3.2	3.8	10.4	48	.9
F_1	81	3.4	4.1	10	-	.9

[z] CL = culm length; IL = internode length for last three internodes, "last" is just below the peduncle; P = peduncle length; FL = inflorescence length.
[y] ½s = ½ sib to the upright parent (64-20) used in inheritance study; fs = full sib to the mound parent (35-9); ap = another prostrate plant, not necessarily related to the prostrate parent.

Table 2. Morphological variation of growth habits in Fountain Grass.

Plant	LC[z]	SC	SP/cm	SW (gm)	FC
upright					
64-20	146A	197B	19	.11	100
F_1	146A	197B	--	.09	100
64-3 ½s[y]	--	199A	25	.11	50-100
F_1	--	199A	--	.12	>100
mound					
35-9	138A	199A	29	.16	>100
F_1	138A	199A	--	--[x]	60
35-20 fs[y]	--	199B	35	.20	>100
F_1	--	199A	--	.14	50-100
prostrate					
14-2	138A	164A	34	.10	<50
F_1	138A	165B	--	.08	<50
22-13ap[y]	138A	164B	26	.16	50-100
23-9 ap[y]	138A	164B	34	.13	50-100
F_1	138A	165B	--	.17	50
dwarf					
9094	137A	197C	18	.08	50-100
F_1	137A	197B	--	.18	50-100

[z] LC = leaf color; SC = seed color, based on Royal Horticultural Society's Colour Chart; SP/cm = spikelets per cm of inflorescence; SW = seed weight, gm/100 seed; FC = flowering culms per plant.

[y] ½s = ½ sib to the upright parent (64-20) used in inheritance study; fs = full sib to the mound parent (35-9); ap = another prostrate plant, not necessarily related to the prostrate parent.

[x] too few seeds to weight.

Table 3. Morphological variation of growth habits in Fountain Grass.

Plant	DFL[z] 1991	1992	LL	LW	CA[0]
upright					
64-20	8-30	9-17	73	0.7	75
F_1	9-8	9-5	49	0.7	68
64-3 ½s[y]	8-20	8-30	54	0.8	70
F_1	8-24	--	50	0.6	65
mound					
35-9	8-16	8-20	58	1.0	60
F_1	8-27	9-6	38	0.9	60
35-20 fs	8-18[x]	--	58	1.0	60
F_1	8-25[x]	--	35	0.8	60
prostrate					
14-2	8-21	8-20	32	0.8	45
F_1	8-21	8-12	36	0.8	50
22-13	--	--	38	0.7	45
23-9 ap	8-21	--	42	0.9	45
F_1	8-24	--	35	0.8	60
dwarf					
9094	8-16	8-20	33	0.4	68
F_1	8-7	8-25	35	0.4	60

[z] DFL = date of flowering or 50% anthesis; LL = leaf length; LW = leaf width; FL = inflorescence length; CA = average culm angle from horizontal ground zero;

[y] ½s = ½ sib to the upright parent (64-20) used in inheritance study; fs = full sib to the mound parent (35-9); ap = another prostrate plant, not necessarily related to the prostrate parent.

[x] this plant and its progeny had abnormal anthers that never appeared to open or shed pollen.

APPENDIX 3

SEED GERMINATION OF FOUR GROWTH HABITS, F_1 AND F_2 PROGENY IN FOUNTAIN GRASS, 1989-1992.

Inflorescences were bagged prior to stigma emergence. For crosses, the appropriate pollen was applied when receptive stigmas were observed,and the heads were immediately rebagged. Inflorescences were collected approximately one month after pollination, the seed was hand cleaned and weighed. In early March, seeds were placed on moist blotter paper in 75cm x 75 cm x 25 cm boxes which were covered with clear plexiglass. The boxes were placed under cool white fluorescent lights with 16 hr days.

A seed was counted as germinated when the radicle and coleoptyle were both visible. The number placed per line varied from 2 to 94, depending on seed set, the average being 51. The seed were then transplanted into cell packs in the greenhouse and then in May moved to the field for the inheritance and isozyme studies reported in Chapters One and Two.

Table 1. % Seed Germination for four growth habits and progeny.

Genotype	Year	Germ %
prostrate		
op;gh[z]		
9014(parent)	1989	89
9015(full sib)	89	64
op;arb		
½ sibs of parent		
9173	90	0
9174	90	0
F_1		
2010,self	91	73
2010A[y]	91	38
2010C	91	86
2010D	91	78

[z] op;gh = open pollinated;greenhouse; op;arb = open pollinated;arboretum.

[y] Letters represent male parent in crosses
A = upright
B,G = ½ sib of prostrate
C = dwarf
D = mound
F = prostrate
J,I = ½ sibs of upright

Table 1. % Seed Germination for four growth habits and progeny.

Genotype	Year	Germ %
prostrate cont'd.		
F_1 repeats		
2010SR,self	92	90
2010AR	92	67
2010S1C[x]	92	12
2010DR	92	63
F_2		
2010-F2	92	49
2010A-F2	92	90
2010C-F2	92	92
2010D-F2	92	90
Mean Germination of prostrate: 61%		
mound		
9035(parent)	89	93
9034(full sib)	89	100
full sibs of mound		
9182	90	1
9183	90	29
9184	90	6
F_1		
2013,self	91	38
2013A	91	93
2013C	91	94
2013F	91	4
F_1 repeats		
2013,self	92	86
2013CR	92	90
2013S1F[x]	92	31
2013JR	92	88
F_2		
2013-S2	92	22
2013A-F2	92	76
2013B-F2	92	84
2013C-F2	92	82
Mean Germination Mound = 60%		

[z] op;gh = open pollinated;greenhouse; op;arb = open pollinated;arboretum.

[y] Letters represent male parent in crosses
A = upright
B,G = ½ sib of prostrate
C = dwarf
D = mound
F = prostrate
J,I = ½ sibs of upright

[x] F_1 substituted for parent

Table 1. % Seed Germination for four growth habits and progeny.

Genotype	Year	Germ %
upright		
op;arb		
9063 full sib	89	78
9064 parent	89	85
½ sibs of u parent		
9188	90	35
9190	90	7
9191	90	16
9192	90	0
F_1		
2017,self	91	33
2017C	91	90
2017D	91	90
2017F	91	69
2017I	91	73
F_1 repeats		
2017SR,self	92	90
2017CR	92	45
2017DR	92	84
2017FR	92	84
F_2		
2017-S2,self	92	13
2017C-F2	92	72
2017D-F2	92	78
2017F-F2	92	98
Mean Germination Upright = 60%		
dwarf		
Selfed;gh		
9094 parent	89	31
F_1		
2019,self	91	90
2019D	91	100
2019G	91	83
2019I	91	100

[z] op;gh = open pollinated;greenhouse; op;arb = open pollinated;arboretum.

[y] Letters represent male parent in crosses
A = upright
B,G = ½ sib of prostrate
C = dwarf
D = mound
F = prostrate
J,I = ½ sibs of upright

[x] F_1 substituted for parent

Table 1. % Seed Germination for four growth habits and progeny.

Genotype	Year	Germ %
dwarf cont'd.		
F_1 repeats		
2019SR	92	41
2010S1C[x]	92	94
2019J	92	66
2019DR	92	36
F_2		
2019S2,self	92	78
2019DF2	92	90
2019GF2	92	82
2019IF2	92	96
Mean Germination Dwarf		76%
Mean Germination Prostrate		61%
Mean Germination Mound		60%
Mean Germination Upright		60%

There was no significant difference between means of seed germination of the 4 growth habits.

[z] op;gh = open pollinated;greenhouse; op;arb = open pollinated;arboretum.

[y] Letters represent male parent in crosses
A = upright
B,G = ½ sib of prostrate
C = dwarf
D = mound
F = prostrate
J,I = ½ sibs of upright

[x] F_1 substituted for parent

APPENDIX 4

SEED SET DATA FROM SELF, OPEN AND CROSS POLLINATIONS OF FOUNTAIN GRASS 1990-1992

All plants were grown at the Minnesota Landscape Arboretum or in the research area at the St Paul campus. Inflorescences were bagged prior to stigma emergence. For crosses, the appropriate pollen was applied as stigmas emerged and the heads were immediately rebagged. Approximately one month after bagging, the heads were removed, seed was hand cleaned and weighed. Inflorescences that had not been bagged were randomly selected for the open pollinations. Five observations were used to compute each mean in Table 1 and 2. Complete data is listed in Table 3.

Table 1. Comparison of means in seed set (in gm) of self,open and cross pollinations within four growth habits of Fountain Grass.

S/F[z]	p[y]	m	u	d	Grand Mean
S_1	.17 a[x]	.07 b	.21 ab	.04 a	.12 ab
S_2	.03 a	.01 b	.05 a	.03 a	.03 a
S_3	.006 a	.01 b	.05 a	.004 a	.02 a
F_1	.20 a	.31 a	.35 b	.04 a	.23 b
F_2	.20 a	.11 ab	.02 a	.18 b	.13 ab
F_3	.03 a	.004 b	.03 a	.06 ab	.03 a
OP	.20 a	.62 c	.20 ab	.18 b	.29 b

[z] S = self; F = crosses to other three growth habits; OP = open pollinated.
[y] p = prostrate; m = mound; u = upright; d = dwarf.
[x] means followed by different letters are significantly different within columns at P = .05, LSD.

The F_1 seed set and open pollinations when combined for all four parents had significantly higher seed set than any selfs or F_2 or F_3 lines.

Table 2. Comparison of means in seed set (in gm) of self,open and cross pollinations between four growth habits.

S/F[z]	p[y]	m	u	d	Grand Mean
S_1	.17 a[x]	.07 a	.21 a	.04 a	.12
S_2	.03 ab	.01 b	.05 a	.03 ab	.03
S_3	.006 a	.01 a	.05 a	.004 a	.02
F_1	.20 ab	.31 b	.35 b	.04 a	.23
F_2	.20 a	.11 a	.02 a	.18 a	.13
F_3	.03 a	.004 a	.03 a	.06 a	.03
OP	.20 b	.62 a	.20 b	.18 b	.29

[z] S = self; F = crosses to other three growth habits; OP = open pollinated.
[y] p = prostrate; m = mound; u = upright; d = dwarf.
[x] means followed by different letters are significantly different between columns at P = .05, LSD.

The mound open pollinations had significantly more seed set than the open pollinations in the other three parents.

Table 3. Seed set data for four growth habits, in selfs, crosses and open pollinations. 1990-1992.

GROWTH HABIT	BAGGING DATE	SELF,CROSS,OPEN	SEED WT GM
PROSTRATE		SELFS	
S_1			
14-2	8-26-90	self;Arb	.28
	"	"	.25
	"	"	.14
	8-31-90	"	.13
	8-26-90	"	.05
	"	"	.05
	8-5-90	"	.23
14-2	1991	selfs	
9 not cleaned			
S_2			
2010-10	8-28-91	S_2	<.01
2010-10	"	"	0
2010-10	"	"	.05
2010-9	8-13-91	"	<.01
2010-7	8-17-91	"	.06
2010-7	8-21-91	"	<.01
2010-11	8-17-91	"	.08
2010-13	8-28-91	"	.03
10left			
S_3			
2010S2-9	8-21-92	S_3	0
"	"	"	0
"	"	"	0
2010S2-4	8-21	"	0
"	4-92	"	0
PROSTRATE		CROSSES	
F_1			
14-2	8-26-90	x m	.13
	"	x m	.28
	8-31-90	x d	.12
	9-6-90	x u	.18
	8-31-90	x d	.37
14-2	8-8-91	x m	.07
	8-24-91	x u	.07
	8-4-91	x u ½sib	.13
14-2	8-15-92	x m	.07
F_2			
2010D-8[z]	8-7-91	F_2	.11
2010D-25	8-7-91	"	.08
2010D-25	7-31-92	"	.20
2 heads left			

[z] Letters represent male parent in crosses: A = upright, B,G = ½ sib of prostrate, C = dwarf, D = mound, F = prostrate, J,I = ½ sibs of upright

GROWTH HABIT	BAGGING DATE	SELF,CROSS,OPEN	SEED WT GM
PROSTRATE		CROSSES	
2010C-5	8-7-91	F_2	.52
2010C-6	"	"	.08
2010C-6	9-13-91	"	0
3 heads left			
2010B-1	8-7-91	F_2	.03
2010B-11	"	"	.33
4 heads left			
2010A-2	9-13-91	F_2	0
2010A-10	8-7-91	"	.20
2010A-11	"	"	.19
1head left			
F_3			
2010AF2-6	8-3,6,21-92	F_3	2 zero;2<.01
2010DF2-27	8-4-92	"	.15
"	8-6-92	"	<.01
2010CF2-10	8-6-92	"	5 zero
2010CF2-14	8-6-92	"	0
"	8-3-92	"	.04
"	8-6-92	"	.01
"	8-12-92	"	.02
"	8-3-92	"	.04
"	8-3-92	"	.01
"	"	"	.33
PROSTRATE		OPEN POLLINATIONS	
14-2	9-4-90	openArb	.22
	9-4-90	"	.20
	"	"	.18
	"	"	.13
	"	"	.27
	"	"	.07
MOUND		SELFS	
S_1			
35-9	8-6-90	selfArb.	.29
35-9	"	"	.02
35-9	8-18-91	"	.01
35-9	8-4-91	"	.01
"	8-15-91	"	0
35-9	8-16-91	"	.09
"	8-15-91	"	.04
"	8-20-91	"	.02
"	8-18-91	"	.01
"	8-4-91	"	.01
"	8-18-91	"	.13
"	8-20-91	"	.02
"	8-24-91	"	0

GROWTH HABIT	BAGGING DATE	SELF,CROSS,OPEN	SEED WT GM
MOUND		SELFS	
S_2			
2013-1	8-29-91	S_2	.04
2013-1	"	"	0
2013-6	9-13-91	"	0
2013-9	8-25-91	"	0
2013-10	cant read	"	0
2013-11	"	"	0
2013-2	8-13-91	"	.01
2013-5	8-17-91	"	0
2013-3	8-28-91	"	.02
2013-4	9-12-91	"	0
2013-8	8-28-91	"	.02
2013-8	8-13-91	"	3seed
2013-13	8-22-91	"	0
2013-2	8-25-91	"	.07
S_3			
2013S2	8-6-92	S_3	0
"	8-3-92	"	<.01
"	8-21-92	"	.02
MOUND		CROSSES	
F_1			
35-9	9-6-90	x p	.03
35-9	9-4-90	x u	.37
"	9-4-90	x d	.21
35-9	8-16-91	x d	.13
"	8-11-91	x d	.52
"	8-20-91	x u	0
F_2			
2013F-2	7-31-92	F_2	<.01
"	8-16-92	"	.22
2013C-2	8-8-91	F_2	.05
2013C-4	"	"	.01
2013C-6	"	"	.09
2013C-9	"	"	0
2013C-7	"	"	.06
2013C-12	9-13-91?	"	0
2013B-8	8-8-91	F_2	.29
2013B-13	"	"	<.01
2013B-2	"	"	.33
2013B-4	"	"	.49
2013B-14	"	"	<.01
2013A-9	8-10-91	F_2	.28
2013A-11	"	"	.02
2013A-4	"	"	.29
2013A-12	"	"	.27
2013A-14	"	"	.18
2013A-8	"	"	.09

GROWTH HABIT	BAGGING DATE	SELF,CROSS,OPEN	SEED WT GM
MOUND		CROSSES	
F_3			
2013BF2	8-3-92	F_3	.02
"	"	"	0
"	8-6-92	"	2,each<.01
2013AF2-18	8-3-92	"	2 zero
"	8-21-92	"	2, .02
2013CF2-18	7-31-92	"	0
"	"	"	3, <.01
2013CF2-22	7-31-92	F_3	0
2013CF2-22	9-6-92	"	1 seed
"	8-6-92	"	.04
"	"	"	0
"	7-31-92	"	<.01
MOUND		OPEN POLLINATIONS	
35-9	9-12-90	openArb.	.29
"	"	"	1.15
"	"	"	.83
"	"	"	.34
"	9-12-90	openArb.	.53
"	"	"	.12
UPRIGHT		SELFS	
S_1			
64-20	9-10-90	self;Arb	.01
"	9-12-90	"	0
64-20	8-11-91	self;Arb	.88
"	8-27-91	"	.01
"	8-24-91	"	.16
"	8-12-91	"	.35
"	8-20-91	"	.09
3 heads left			
64-20	8-24-92	self;Arb	3 zero
"	"	"	2heads each<.01
S_2			
2017-1	8-28-91	S_2	0
2017-2	"	"	.04
2017-3	"	"	<.01
2017-3	8-25-91	"	.07
2017-2	8-25-91	"	.11
2017-3	8-10-91	"	.03
2017-2	8-25-91	"	.21
2017-1	"	"	.10
2017-3	8-28-91	"	<.01
2017-1	8-21-92	"	.06
2017-1	"	"	.02

GROWTH HABIT	BAGGING DATE	SELF,CROSS,OPEN	SEED WT GM
UPRIGHT		SELFS	
S_3			
2017S2-9	8-3-92	S_3	.07
"	8-21-92	"	.15
2017S2-3	8-6-92	"	.01
"	8-3-92	"	.04
"	8-6-92	"	0
"	8-3-92	"	0
UPRIGHT		CROSSES	
F_1			
64-20	8-31-90	x d	.36
"	8-28-90	x p	.37
"	8-20-90	x d	.27
	8-28-90	x m	.52
	9-6-90	x p	.16
	9-6-90	x m	.20
64-20	8-24-91	x d	.19
"	8-20-91	x p	.17
"	8-16-91	x p	.25
64-20	8-4-91	x m	.27
"	8-12-91	x m	.48
F_2			
2017I-36	9-4-91	F_2	0
2017I-4	"	"	<.01
2017-42	8-10-91	"	0
2017I-5	"	"	0
2017I-46	"	"	.07
2017I-36	"	"	1 seed
2017I-7	8-10-91	"	.04
2017I-38	9-4-91	"	0
2017I-27	8-10-91	"	.07
2017I-41	9-4-91	"	0
2017F-4	8-10-91	F_2	.09
2017F-2	9-4-91	"	0
2017F-5	8-10-91	"	.02
2017F-2	"	"	0
2017F-3	"	"	<.01
2017F-1	"	"	.72
2017D-11	9-4-91	F_2	0
2017D-11	"	"	1seed
2017D-5	8-10-91	"	.05
2017D-12	8-10-91	"	.23
3 heads left			
2017C-4	8-10-91	F_2	.01
2017C-2	"	"	0
2017C-3	"	"	0
2017C-14	9-10-91	"	0
2017C-14	9-4-91	"	0
2017C-14	"	"	0
2017C-14	"	"	0
2017C-9	8-10-91	"	0

GROWTH HABIT	BAGGING DATE	SELF,CROSS,OPEN	SEED WT GM
UPRIGHT		CROSSES	
2017C-22	7-23-91	F_2	0
2017C-22	"	"	.04
2017C-21	9-4-91	"	0
2017C-5	8-10-91	"	.02
2017C-22	8-6-92	F_2	3zeros;+ 1 with 1seed
"	"	"	.02
F_3			
2017FF2-11	8-3-92	F_3	3 zeros
2017FF2-1	8-6-92	"	.09
"	8-12-93	"	.08
"	8-3-92	"	.20
2017DF2-14	8-3-92	F_3	.05
"	"	"	0
"	"&21	"	2 zeros
2017CF2-6	8-4-92	F_3	.08
"	8-21-92	"	0
"	8-4-92	"	0
UPRIGHT		OPEN POLLINATIONS	
64-20	9-12-90	open;Arb	.21
"	"	"	.46
"	"	"	.09
"	"	"	.26
"	"	"	.06
"	"	"	.08
DWARF		SELFS	
S_1			
9094	8-7-90	self;Arb.	.01
"	"	"	.12
"	"	"	.01
"	8-26-90	"	.09
"			
"	8-11-91	selfs	0
"	7-22-91	"	0
"	8-11-91	"	.01 3seed
"	7-22-91	"	0
"	8-27-91	"	0
"	8-4-91	"	.02
"	8-20-91	"	0
"	8-15-92	selfs	0
"	"	"	0
"	"	"	0
"	"	"	0
"	"	"	0
"	"	"	0
"	8-20-92	"	5 zeros
"	8-26-92	"	5 zeros
"	8-29-92	"	4 zeros

GROWTH HABIT	BAGGING DATE	SELF,CROSS,OPEN	SEED WT GM
DWARF		SELFS	
Bed1	8-5-92	S_1	5 seed
"	8-5-92	"	3zero
"	8-15-92	"	.05
"	"	"	2 zero
"	8-20-92	"	5 seed; .01
"	"	"	1 seed
"	"	"	.02
"	8-23-92	"	.01
"	"	"	2 zero
"	"	"	.01
S_2			
2019-16	8-10-91	S_2	<.01
2019-2	8-13-91	"	.01
2019-11	8-10-91	"	.03
2019-15	"	"	.03
2019-5	7-23-91	"	.05
2019-3	8-10-91	"	.04
2019-5	8-17-91	"	.05
2019-9	8-21-91	"	0
2019-4	8-22-91	"	<.01
2019-5	7-23-91	"	<.01
2019-12	7-23-91	"	.04
2019-17	"	"	.02
2019-14	8-10-91	"	<.01
2019-13	"	"	.07
2019-19	7-23-91	"	.14
2019-12	7-31-92	S_2	2/1 seed each
2019-13	"	"	.08
"	"	"	.05
"	"	"	.07
"	"	"	<.01
"	"	"	1 seed
2019-12	8-6-92	"	2/5 seed each
2019-9	"	"	1 seed
2019-10	"	"	2, <.01
"	"	"	.03
"	"	"	0
2019-10	8-6-92	"	.02
S_3			
2019S2	8-3-92	S_3	6 seed
"	"	"	0
DWARF		CROSSES	
F_1	8-26-90	x m	.16
"	8-20-91	x m	.01
"	8-4-91	x u ½ sib	0
"	8-11-91	x u ½ sib	.01
"	8-4-91	x m	.01

GROWTH HABIT	BAGGING DATE	SELF,CROSS,OPEN	SEED WT GM
DWARF		CROSSES	
F_2			
2019I-2	8-10-91	F_2	.01
2019I-2	"	"	.02
2019I-1	"	"	.10
2019I-2	"	"	.09
2019I-1	"	"	<.01
2019G-3	8-10-91	F_2	.35
2019G-1	"	"	.05
2019G-5	"	"	.51
4 left			
2019D-7	8-10-91	F_2	.49
2019D-3	"	"	.07
2019D-5	"	"	.07
2019D-4	"	"	.10
2019D-2	9-4-91	"	0
F_3			
2019GF2-28	8-3-92	F_3	.11
"	8-6-92	"	.15
3 more heads			
2019GF2-23 not cl			
2019DF2-18	8-6-92	F_3	.02
"	8-3-92	"	2 zeros
"	8-6-92	"	<.01
2019IF2-23	8-21-92	F_3	<.01 for each, 3
DWARF		OPEN POLLINATIONS	
9094	9-4-90	openArb	.20
"	"	"	.17
"	"	"	.16
"	"	"	.21
"	"	"	.16
"	"	"	.17

APPENDIX 5

ADDITIONAL FIGURES OF PGI-2 ISOZYME SEGREGATION
IN FOUNTAIN GRASS

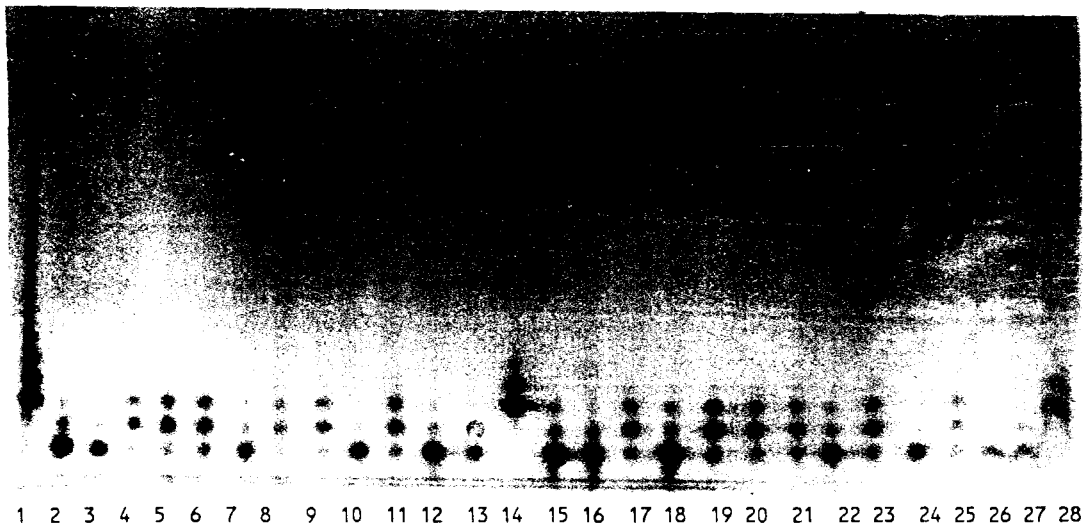

Figure 1. F_1 segregation of 13 SS and 12 FS individuals from a cross between the dwarf, SS, female parent, and an upright FS, male parent. Controls in lanes 1, 14 and 28 are FF.

APPENDIX 6

PRELIMINARY IN VITRO POLLEN GERMINATION OF PENNISETUM ALOPECUROIDES (L.) Spreng. AND PENNISETUM SETACEUM (Forsk.)Chiov.

Pollen was collected from April through July 1990 from plants grown in the greenhouse. Many different genotypes were tested but only those with a minimum of 5 counts are reported.

Pollen was collected from individual plants into glassine pollination bags at approximately 1000 hr as the anthers dehisced; afternoon collections were not successful. The pollen was taken to the lab where it was placed on a glass slide. The slide was placed on moist filter paper in a petri dish and covered. The pollen was left to hydrate for 20-30 minutes. Pollen was then dusted onto approximately 0.1 ml of solution and sealed in a depression slide at room temperature. The germination solution was 25 g kg sucrose and 100 mg ml boric acid in distilled water.

Germination counts were made within 24 hours. A pollen grain was considered to be germinated when the length of the pollen tube was equal to or longer than the diameter of the grain. A minimum of 300 grains per slide were counted. A pollen grain that germinated was considered to be viable.

Table 1. Pollen viability of ten genotypes of Fountain Grass.

Genotype & Growth Habit	# Slides	Germination % Mean	Range	
1996 upright[z]	9	1	0	2
2001 upright	11	10	0	43
413373	8	22	0	59
1980 F_1 prostrate	25	21	1	61
1981 "	5	12	0	28
1982 "	8	22	1	59
1985 "	5	1	0	3
1987 "	7	24	0	59
1989 "	11	5	0	19
2005 prostrate	8	16	1	38

[z] 1996 and 2001 were ½ sibs of the upright parent used in the inheritance study, both from Bluemel Nursery; see Appendix 1 for source of 413373; 1980-1989 are F_1 open pollinated progeny from 90190 Germplasm Lab. 2005 is a plant from the original seed lot 90190 from the Germplasm Lab, see Appendix 1.

Another species Pennisetum setaceum, Crimson Fountain Grass, was also tested for viability. This pollen was handled as outlined above, however pollen of this species was found to be viable even after 5 days. The pollen was simply held in the glassine collection bags at room temperature and tested for several consecutive days. Approximately 50% of the pollen of this species is empty, not filled and not viable. The germination counts below are based on filled grains only.

Table 2. Pollen Viability of Crimson Fountain Grass, Pennisetum setaceum.

Pollen Age (days)	#slides	Germination % Mean	Range	
Fresh	14	19	0	32
1	5	3	1	7
2	1	1	0	1
3	3	<1	0	<1
4	2	<1	0	<1
5	2	<1	0	<1

APPENDIX 7

PRELIMINARY COLD HARDINESS DATA FOR FOUNTAIN GRASS 1990-1991

Cold Evaluation #1 NOVEMBER 1990

Crowns from several genotypes of Pennisetum alopecuroides were dug from the arboretum, cut back, divided and packed with potting soil into small plastic cylinders that fit the freezer block. Three groups of 48 crowns each were prepared to be tested in November, January, and April. The November crowns were tested just after dividing, and the others were held in the cold frame (with minimal protection) until the date indicated.

The cylinders with crowns were placed in the freezer where the temp was 3^0 C. The temperature was then lowered 2^0 per hour and held at the designated temperature for ½ hr after which the crowns were removed and held at 4^0 for 12 hours. The treated crowns and controls were then planted in 3" pots and placed in the greenhouse. Controls (also in small plastic cylinders) were held in the cold frame with minimal protection prior to planting.

The prostrate genotypes had eight replicates at each temperature and eight controls. The upright and tawny mound had four replicates at each temperature and four controls.

Table 1. Crown regrowth after freezer tests November 1990.

	Temperature, C			
Genotype	-10	-18	-26	Controls
prostrate[z]	3	0	0	6
upright	0	0	0	4
tawny mound	0	1	0	4

[z] prostrate genotypes tested were # 14-2 the parent in the inheritance study, and 22-13, and 35-10, two other unrelated prostrate genotypes. upright genotypes tested were 64-20, the upright parent in the inheritance study, 64-3, 2002, and 1996, all ½ sibs of 64-20; the tawny mound is a separate genotype, see Appendix 1.

Table 2. Crown regrowth after freezer tests in January 1991.

Genotype	Temperature, C -10	-16	-22	Controls[y]
prostrate[z]	2	2	0	1
upright	0	0	0	1
tawny mound	1	1	0	1

Table 3. Crown regrowth after freezer tests in April 1991.

Genotype	Temperature, C -3	-6	-9	Control
prostrate[z]	0	0	0	0
upright	0	0	0	0
tawny mound	0	0	0	0

[z] prostrate genotypes tested were # 14-2 the parent in the inheritance study, and 22-13, and 35-10, two other unrelated prostrate genotypes. upright genotypes tested were 64-20, the upright parent in the inheritance study, 64-3, 2002, and 1996, all ½ sibs of 64-20; the tawny mound is a separate genotype, see Appendix 1.

[y] -24° F air temp on December 23, 1990 probably damaged crowns prior to testing.

Cole Evaluation #2 April 1991.

A prostrate genotype, (# 22-9, not related to the parent in the inheritance study) was dug from the arboretum on April 2, 1991. Crowns were divided, packed with potting soil into plastic freezer cylinders and placed in the freezer on April 3, where the temp was 3^0 C. The temperature was then lowered 2^0 per hour and held at the designated temperature for ½ hr after which the crowns were removed and held at 4^0 for 12 hours. The treated crowns and controls were then planted in 3" pots and placed in the greenhouse. Fourteen controls (also in small plastic cylinders) were held in the cold frame with minimal protection prior to planting. Six replicates were tested for each temperature.

Table 1. Crown regrowth of a prostrate genotype subjected to 8 different low temperatures.

	Temperature C								
genotype	-3	-5	-7	-9	-11	-13	-15	-17	Controls
22-9	6	6	6	6	5	4	1	0	14

The LD 50 for this genotype appears to be -14° C.

Cold Evaluation #3 NOVEMBER 1991.

Three genotypes were dug from the St Paul plots on November 11, 1991. Crowns were divided and packed with potting soil into plastic freezer containers and placed in the freezer for testing, as described above, or into the cold frame, with minimal protection, to be tested as soon as possible, before normal weather conditions killed the crowns.

Table 1. Crown regrowth of the dwarf after freezer tests November, 1991.

	Temperature C							
genotype	-5	-7	-9	-11	-13	-15	-17	Controls
dwarf[z]	6[y]	5	4	2	0	0	0	17

[z] F_1 dwarf genotype from the inheritance study
[y] 6 replicates at -5, all others temps had 7 reps; 17 controls

The LD 50 appears to be approximately -10^0 C for this genotype.

Table 2. Crown regrowth of the F_1 tawny mound genotype after freezer tests, December 1991.

	Temperature C						
genotype	-7	-9	-11	-13	-15	-17	Controls
tawny mound	7[z]	2	2	0	0	0	11

[z] 8 replicates per temp; 14 controls

The LD 50 appears to be approximately -8^0 C for this genotype.

Table 3. Crown regrowth of the F_1 upright genotype 2016[z] after freezer tests, December 1991.

	Temperature C							
genotype	-5	-7	-9	-11	-13	-15	-17	Controls
upright	6[y]	4	3	2	0	0	0	11

[z] a F_1 ½ sib of the upright used in the inheritance study
[y] 7 replicates per temp except only 6 at -5; 12 controls

LD 50 appears to be approximately -8° C for this genotype.

APPENDIX 8

FOUNTAIN GRASS SEED GERMINATION AFTER COLD TREATMENTS

Seed of five genotypes of Pennisetum alopecuroides was subjected to low temperatures in November 1990 or January 1991. Dry seed, 20 per treatment, was lightly wrapped in tissue (to hold it in place) in plastic cylinders and placed in the freezer at 3° C. Temperatures were lowered 2° C per hour and held at the indicated temperatures for ½ hour, after which the seed was removed. Twenty controls per genotype and the treated seeds were then placed on moist filter paper in petri dishes at room temp under cool white fluorescent light with 16 hr days. Seeds were counted as germinated when the radical and coleoptile were both visible.

Table 1. Germination of five fountain grass genotypes subjected to low temperatures in Nov. 1990 or Jan. 1991. Each treatment consisted of 20 seeds.

Genotype	Temperature, C				Control[z]		Mean of each
	Nov.1990		Jan.1991				
	-18	-26	-16	-22	Nov	Jan	genotype
22-13 p[y]	14	19	18	19	16	20	17.7 a[x]
14-2 p	12	13	17	17	8	19	14.3 a
35-10 dp	12	10	12	5	10	13	10.3 b
64-3 u	13	13	11	14	5	17	11.5 b
70-5 tm	11	9	19	18	11	20	14.7 a

[z] dormancy may have affected germination.
[y] p = prostrate; dp = dwarf prostrate a small plant from the prostrate line; u = upright; tm = tawny mound; see Appendix 1.
[x] means followed by a different letter are significantly different at P ≤.05, LSD.